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DRINKING WATER CRITERIA DOCUMENT FOR METHOXYCHLOR

Prepared for

OFFICE OF DRINKING WATER

Prepared by

Environmental Criteria and
Assessment Office
Cincinnati OH 45268

DRAFT: DO NOT CITE OR QUOTE

NOTICE

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16. ABSTRACT

The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on methoxychlor.
This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of methoxychlor
- Toxicokinetics and human exposure to methoxychlor
- Health Effects of methoxychlor in humans and animals
- Mechanisms of toxicity of methoxychlor
- Quantification of toxicological effects of methoxychlor

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
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LIST OF ABBREVIATIONS

AADI	Adjusted acceptable daily intake
ADI	Acceptable daily intake
bw	Body weight
CNS	Central nervous system
DNA	Deoxyribonucleic acid
ER	Endoplasmic reticulum
HA	Health advisory
LD_{50}	Lethal dose to 50% of recipients
LOAEL	Lowest-observed-adverse-effect level
NOAEL	No-observed-adverse-effect level
ppm	Parts per million
s.c.	Subcutaneous
TLV	Threshold limit value
TWA	Time-weighted average

I. SUMMARY

Methoxychlor, 2,2-bis(4-methoxyphenyl)-1,1,1-trichloroethane, is the p,p'-methoxy analog of 1,1,1-trichloro-2,2-bis(para-chlorophenyl)ethane (DDT) with a molecular weight of 345.65. Some of the physical properties of methoxychlor include a melting point (dimorphic) of 78-78.2°C or 86-88°C and specific gravity of 1.41. Methoxychlor has low solubility in both freshwater and salt-water (0.12 mg/ℓ at 25°C and 0.05 mg/ℓ at 20°C, respectively).

Studies with experimental animals indicate that methoxychlor is rapidly metabolized in the liver to the mono- and bisphenols resulting from O-demethylation and to 2,2-bis(p-hydroxyphenyl)-1,1-dichloroethylene, the dehydrochlorination product of the bisphenol. Other metabolites present in significant quantities include bis(p-hydroxyphenyl)acetic acid and p,p'-dihydroxybenzophenone. Excretion of metabolites into the urine and feces is rapid, generally within 24 hours. Neither the parent compound nor its metabolites appear to be stored to any significant extent in fat or other tissues.

The acute oral LD_{50} of methoxychlor (in a lipophilic vehicle) in rats is ~6 g/kg bw. A toxic dose causes depression of the CNS, progressive weakness, and diarrhea followed by death in 36-48 hours. Subtle biochemical changes in hepatic function, specifically glycogen metabolism, were revealed when much lower single doses of methoxychlor were administered by gavage to rats (0.04-0.64 g/kg bw). Methoxychlor is more toxic than DDT when administered intravenously to rats.

Growth retardation is the most common feature of sublethal methoxychlor poisoning following ingestion. In subchronic and chronic feeding studies in rats, diets containing 500-1000 mg/kg methoxychlor caused slight growth retardation; but this response could not be elicited when treated animals were compared with pair-fed controls signifying a possible organoleptic effect and not a toxic effect. Diets containing 2000 mg/kg methoxychlor resulted in significantly decreased testicular weight in rats. Dogs, rats and swine all manifested distinctly different toxicity patterns following dietary administration of methoxychlor: CNS effects were observed in dogs; chronic nephritis and cystic tubular nephropathy in swine and rats, respectively; fatty changes in the livers of swine; marked testicular atrophy in rats and swine; and mammary gland hypertrophy and hyperplasia in swine. Histopathologic changes in the kidneys, liver or reproductive organs were not observed at dietary levels below 1000 mg/kg.

The evidence from studies with experimental animals and *in vitro* assays indicates that methoxychlor is not a carcinogen, mutagen or teratogen. A number of chronic dietary studies, including an NCI bioassay, have been conducted in mice and rats to determine the carcinogenicity of methoxychlor. The interpretations of the original investigators indicate that methoxychlor was not carcinogenic under the conditions of the assays. Re-examination of histological slides and statistical reanalyses of tumor incidences by an independent pathologist indicated that methoxychlor was carcinogenic in these studies. The conclusions of IARC, NCI and CAG, however, all support the contentions of the original investigators that methoxychlor is not carcinogenic to mice and rats. Recent reviews of the NCI bioassay program recommend that the results be considered neither positive nor negative, but rather inconclusive. Negative results were obtained from a number of mutagenicity assays (some with and some without a metabolic activation system) in bacteria and yeast, from assays of methoxychlor-induced DNA damage in DNA repair-deficient strains, from recessive lethal assays in *Drosphila melanogaster* and in assays of unscheduled DNA synthesis in mammalian cell cultures. A weakly positive increase in cell transformations was obtained in cultured Balb/3T3 cells. In the only teratogenicity study conducted on methoxychlor, fetal abnormalities (wavy ribs) in rats were present only at doses that were toxic to the dams

The estrogenic effects observed in some experimental animals could be due to the presence of contaminants or the biotransformation of methoxychlor to estrogen-like metabolites or both. Ingestion of methoxychlor also enhances the activity of certain hepatic enzymes in the mixed function oxidase (MFO) system in rats. Data on the effects of methoxychlor in humans are limited.

No increased toxicity or carcinogenicity was observed in rats treated with methoxychlor in combination with Aramite, DDT or thiourea, although coadministration of methoxychlor and carbon tetrachloride resulted in DDT-like induced tremors in rats.

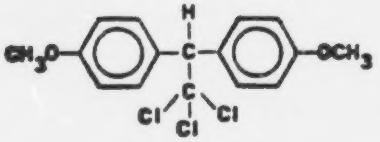
Certain groups such as industrial and agricultural workers or infants breast-fed by mothers with substantial residues of methoxychlor in milk, may have an enhanced risk to methoxychlor exposure.

Several existing guidelines and standards are presented including a maximum pollutant level in drinking water of 0.1 mg/ℓ as specified by U.S. EPA and FDA.

A quantitative risk assessment resulted in the following values: 6.4 mg/ℓ (6.4 mg/day or 0.64 mg/kg bw/day) for a 10 kg child and 22 mg/ℓ (44.8 mg/day or 0.64 mg/kg bw/day) for a 70 kg adult for 1-day HA based upon an oral NOAEL of 640 mg/kg bw to rats producing altered glycogen metabolism. A 10-day HA based upon an oral NOAEL of 50 mg/kg bw/day to rats for 45 days producing retarded growth, for a child is 5.0 mg/ℓ (5.0 mg/day or 0.50 mg/kg bw/day) and for an adult is 18 mg/ℓ (35 mg/day or 0.50 mg/kg bw/day). The recommeded 10-day HAs are based on an oral NOAEL of 2.0 mg/kg bw/day to humans for 4-6 weeks producing no biochemical, hematologic or histopathologic effects of the small intestine, testes or liver. They are 2.0 mg/ℓ (2.0 mg/day or 0.20 mg/kg bw/day) for a child and 7.0 mg/ℓ (14 mg/day or 0.20 mg/kg bw/day) for an adult. An adult life-time AADI based upon an oral NOAEL of 5 mg/kg bw/day to rats for 2 years is 1.7 mg/ℓ assuming the total intake is from water for a 70 kg adult.

II. PHYSICAL AND CHEMICAL PROPERTIES

Chemical Structure



Methoxychlor

Synonyms

2,2-bis(4-methoxyphenyl)-1,1,1-trichloroethane

Marlate (trade name)

2,2-di-α-anisyl-1,1,1-trichloroethane

DMDT

Methoxy-DDT

Chemical Abstracts Service (CAS) Number and Registry of Toxic Effects of Chemical Substances (RTECS) Number:

CAS Number:	72-43-5
RTECS Number:	KJ3675000

Description

Methoxychlor is a pale yellow crystalline solid that is used as an insecticide and is especially effective against mosquito larvae and houseflies (Windholz, 1976). It is recommended for dairy barns (Hawley, 1977).

Physical Properties (Windholz, 1976; Verschueren, 1983)

Molecular Formula:	$C_{16}H_{15}Cl_3O_2$
Molecular Weight:	345.65
Melting Point (Dimorphic) (C°):	78-78.2° or 86-88°

Specific Gravity (25°C): 1.41

Log octanol/water partition coefficient:
3.05 (Coats et al., 1979)
3.31 (Kapoor et al., 1973)
3.68 (Kapoor et al., 1970)
4.30 (Fujita, 1979)

Solubility: (Brooks, 1947)

Solvent	Solubility (g/100 g at 22°C)
Acetone	60
Methylene chloride	60
Dichlorobenzene	40
Cyclohexanone	50
Xylene	40
Methylated naphthalenes	35
Carbon tetrachloride	16
Methanol	<5
Odorless kerosene	2
Water (Richardson and Miller, 1960)	0.1 mg/ℓ at 25°C
Water (Zepp et al., 1976)	0.12 mg/ℓ at 25°C
Natural seawater (salt content = 2.5%) (Liu and Lee, 1975)	0.05 mg/ℓ at 20°C

Impurities

Lamoureux and Feil (1980) have rigorously identified 15 of the 50 impurities in technical grade methoxychlor by comparison of the mass spectra (MS) and nuclear magnetic resonance (NMR) spectra with synthetic samples. Another 10 compounds were identified by mass spectral interpretation. These are presented in Table II-1. The four largest impurities and p,p'-methoxychlor account for 98.88% of the technical grade methoxychlor. The other impurities are present in amounts <0.5% each. Technical methoxychlor contains ~88% of the p,p'-isomer (Martin and Worthing, 1977).

TABLE II-1

Impurities in Technical Methoxychlor

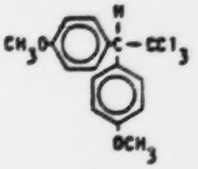
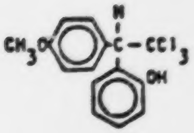
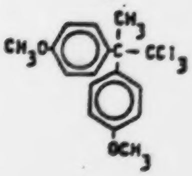
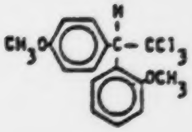
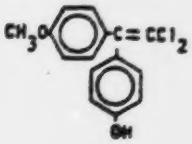
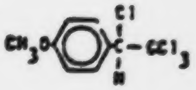
Compound	Relative Amount in Technical Methoxychlor (%)	Evidence for Structure
1.  Methoxychlor	93.1	original sample
2. 		MS analysis
3. 		MS analysis
4. 	3.057	independent synthesis
5. 		MS analysis
6. 	1.430	independent synthesis
7. No proposed structure		MS not interpreted

TABLE II-1 (cont.)

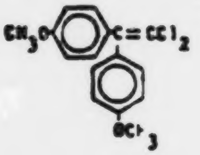
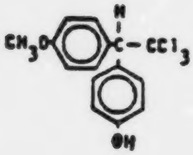
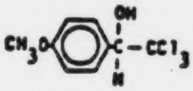
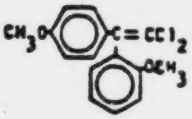
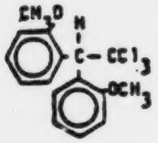
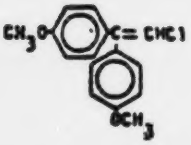
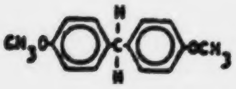
Compound	Relative Amount in Technical Methoxychlor (%)	Evidence for Structure
8.  DMDE	0.761	independent synthesis
9. 	0.531	NMR and MS analysis
10. 	0.363	independent synthesis
11. 	0.161	independent synthesis
12. 	0.087	independent synthesis
13. 	0.072	independent synthesis
14. 	0.057	independent synthesis, present as a mixture
15. No proposed structure	0.035	MS not interpreted

TABLE II-1 (cont.)

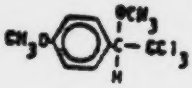
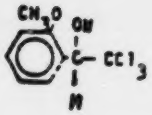
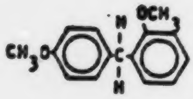
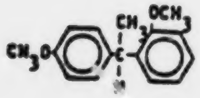
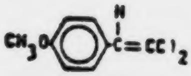
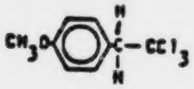
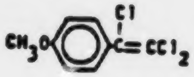
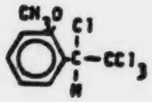
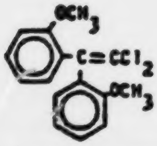
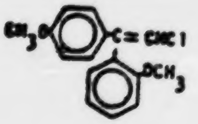
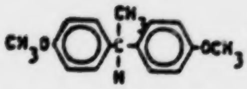
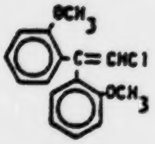
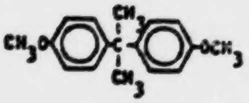
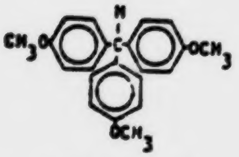
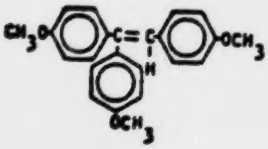
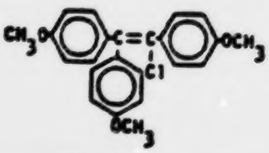
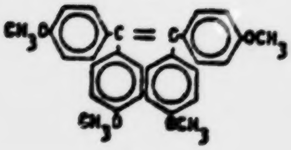
Compound	Relative Amount in Technical Methoxychlor (%)	Evidence for Structure
16. 	0.021	independent synthesis
17. 		
18. 	0.019	present as a mixture, MS analysis
19. 		
20. 	0.012	MS analysis
21. 		
22. 		
23. 	0.011	independent synthesis
24. 	0.006	independent synthesis

TABLE II-1 (cont.)

Compound	Relative Amount in Technical Methoxychlor (%)	Evidence for Structure
25. 	0.004	independent synthesis
26. 	0.003	independent synthesis
27. 	0.002	independent synthesis, MS analysis
28. 		
29. No structure proposed	ND	MS not interpreted
30. 	ND	two isomers, MS analysis
31. 	ND	MS analysis
32. 	ND	MS analysis
33. 	ND	two isomers, MS analysis

ND = not determined

Compounds 32 and 33 were also detected by Mitchell and West (1978) and Mazuch et al. (1975), respectively. Mitchell et al. (1978) and Grant et al. (1976) suggested that compound 33 can photochemically react to form both 3,6-dimethoxy-9,10-bis(4-methoxyphenyl)phenanthrene (34) and 3, 6, 11, 14-tetramethoxydibenzo(g,p)chrysene (35). There is some concern over the possible mutagenic activity of these two compounds (see Carcinogenicity Chapter V).

Summary

Methoxychlor (72-43-5) is the common name for 2,2-bis(4-methoxyphenyl)-1,1,1-trichloroethane. This insecticide is a yellow crystalline solid, slightly soluble in water, soluble in most organic solvents, has a dimorphic melting point (78-72.2°C or 86-88°C) and a specific gravity at 25°C of 1.41. Literature values for log octanol water partition coefficient vary from 3.05 (Coats et al., 1979) to 4.30 (Fujita, 1979). At least 50 impurities exist in the technical formulation, many of which have been identified (see Table II-1).

III. TOXICOKINETICS

No information on the absorption, distribution, metabolism or excretion of methoxychlor in humans was found in the literature searched. Studies with experimental animals indicate that methoxychlor is rapidly metabolized and excreted and that neither the parent compound nor its metabolites are stored to a significant extent in the fat or other tissues.

Absorption

Quantitative data on the absorption of methoxychlor by experimental animals were not found in the literature searched. Absorption of methoxychlor through the gastrointestinal tract and skin can be inferred from methoxychlor's demonstrated systemic toxicity to animals when administered by these routes (Chapter V) and from excretion data (see Excretion). Methoxychlor was found to be more toxic to animals when administered in oil or in food than when administered in aqueous suspension with 1% gum tragacanth (see Vehicle Effect, Chapter V), therefore, it appears that the degree of absorption is affected by the vehicle in which methoxychlor is administered; however, the data are not sufficient to draw firm conclusions.

Distribution

Rats fed methoxychlor in the diet did not accumulate or store this insecticide to a significant extent in their fat or other tissues. In a four-generation study conducted by Adams et al. (1974), rats were fed diets containing different fats or oils at levels similar to those consumed in the United States (20% fat on a dry weight basis; 38% on a caloric basis) and a mixture of chlorinated hydrocarbon insecticides (methoxychlor, DDT, DDD, DDE, dieldrin, lindane, BHC, heptachlor epoxide, Perthane) in the same ratio as that found in the food supply of the United States, but at 200-fold greater concentrations. All insecticides were reference standards or their equivalents in purity as opposed to commercial preparations. The concentration of p,p'-methoxychlor in these diets ranged from 0.078-0.116 ppm (wet wt.). Tissue extracts were analyzed with a gas chromatograph equipped with an electron capture detector. The limit of sensitivity for methoxychlor was not given; for BHC, lindane or dieldrin, the limit of sensitivity was 0.05 ppm in fat and 0.02 ppm in other tissues.

Methoxychlor was not detected in the livers or body fat of adult rats or in the livers, brains, or carcasses of weanlings of any of the four generations fed these diets, regardless of the type of fat in the diet (Adams et al., 1974). Because methoxychlor is an analog of DDT, it is interesting to compare these results with those for DDT and its degradation products. DDT, DDE and DDD, fed in the diet at a combined residue level of 1.6 ppm, were accumulated to a combined residue level of 18 ppm in the body fat of adult rats of each generation (Adams et al., 1974).

The feeding of 20 ppm p,p'-methoxychlor in the diet to three male weanling Wistar rats for 350 days failed to produce detectable levels of methoxychlor in their fat, livers, hearts or brains. The fat content of the diet was not specified; analysis of tissue residues was by gas-liquid chromatography (Villeneuve et al., 1972).

Higher concentrations of methoxychlor in the diet produced low but detectable levels of methoxychlor in the perirenal fat of rats (Kunze et al., 1950). Methoxychlor was not accumulated and was eliminated within 2 weeks after cessation of treatment. Male and female weanling rats were fed 25, 100 or 500 ppm technical p,p'-methoxychlor in the diet for 4-18 weeks. (The fat content of the diet was not specified). No methoxychlor was detected in the fat of rats fed 25 ppm methoxychlor; the limit of sensitivity was 1 ppm. As presented in Table III-1, the feeding of methoxychlor at 100 ppm and 500 ppm in the diet resulted in detectable levels of methoxychlor in the fat in the 9th and 4th weeks of feeding, respectively (Kunze et al., 1950). Two weeks after the treated rats were transferred from test to control diet, methoxychlor could no longer be detected in their fat.

The storage ratio (ratio of ppm methoxychlor in tissue to ppm methoxychlor in the diet) for perirenal fat was about 0.05 when residues were first detected and declined to 0.01-0.03 after 18 weeks of feeding (Kunze et al., 1950). In contrast, rats fed 1 ppm technical DDT in the diet under the same conditions and in the same laboratory accumulated ~15 ppm DDT in perirenal fat after 15 weeks of feeding and 30 ppm after 19 weeks, giving storage ratios for DDT of 15 and 30, respectively (Laug et al., 1950). Four weeks after DDT feeding was terminated, the animals retained in their perirenal fat about 80% of the DDT found in this tissue at the termination of exposure. Much higher dietary levels of methoxychlor (1000, 2500 or 5000 ppm) fed for 6 or 18 weeks to female rats in a reproductive study by Harris et al. (1974) (see Nonteratogenicity Reproductive Effects, Chapter V) resulted in fat storage ratios similar to those reported by Kunze et al. (1950) (i.e., 0.01-0.06).

TABLE III-1

Storage of Methoxychlor in the Perirenal Fat of the Rat[a]

Methoxychlor in Diet (ppm)	ppm Methoxychlor[b] Retained in the Perirenal Fat During Exposure								Cessation of Exposure			
	4 Week		9 Week		13 Week		18 Week		2 Week		4 Week	
	M	F	M	F	M	F	M	F	M	F	M	F
0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
100	ND	ND	4	7	3	4	1	1	ND	ND	ND	ND
500	36	17	26	200	19	14	16	14	ND	ND	ND	ND

[a] Source: Kunze et al., 1950

[b] Each figure represents the average of 2 animals with the exception of the control (0.0 ppm methoxychlor in diet) where average represents 8 animals. All control animals were killed at 18 weeks. The limit of sensitivity for quantification of methoxychlor in fat was 1 ppm (i.e., 1 μg/g).

ND = not detected

Hodge et al. (1952) measured tissue concentrations of methoxychlor in a few rats during the latter part of a 2-year feeding experiment on the chronic effects of 25, 200 or 1600 ppm methoxychlor in the diet (see Effects of Chronic Exposure, Chapter V). The storage ratios of methoxychlor in adipose tissue were ~0.15, 0.02 and 0.01 for the low, intermediate and high dosage levels, respectively. These figures are similar to those reported by Kunze et al. (1950). Storage ratios for kidney were 0.01 and 0.002 for the intermediate and high dosage levels, respectively. For kidney at the low dosage level and for liver and brain at all dosage levels, tissue concentrations were very low and were not quantitated precisely. Dogs given daily doses of 20-300 mg/kg bw for 1 year also had low concentrations of methoxychlor in their tissues (Hodge et al., 1952).

Metabolism

Little specific information on the metabolism of methoxychlor by mammals was published before 1970. Smith et al. (1946) reported that rabbits intubated with 200 mg methoxychlor/kg bw/day in olive oil until death (4-15 doses) excreted some organic chlorine (not further identified) in their urine. Woodward et al. (1948) reported that rats fed methoxychlor in the diet did not excrete the parent compound or bis(p-methoxyphenyl)acetic acid, a metabolite expected by analogy with the metabolism of DDT, in the urine. Other possible metabolites were not investigated.

Following intravenous injection of a saline-lecithin oil emulsion of ^{14}C-ring-labeled methoxychlor at a dose of 3 mg/kg bw to adult male rats, Weikel (1957) found that the radioactive compounds excreted in urine, feces and bile were much more water-soluble than parent methoxychlor. From the behavior of these metabolites on ion exchange columns, Weikel (1957) concluded that they were strongly acidic anionic compounds. The identity of the metabolites was not determined, but they were nontoxic to flies when assayed by the residue contact method.

Studies by Laug and Kunze (1951) and Weikel and Laug (1958) have been widely cited as evidence that the liver is the major site of metabolism of methoxychlor and that metabolism results in detoxication. In these studies, rats were treated with sufficient doses of carbon tetrachloride to cause liver damage and were concurrently or subsequently given methoxychlor as a single intravenous injection. In the first study, published only as an abstract, the authors reported that carbon tetrachloride treatment resulted in an apparent increase in the toxicity of methoxychlor (see Effects of Acute Exposure, Chapter V) and in the accumulation of methoxychlor in abdominal fat and liver in amounts 10-100 times higher than in tissues of rats given methoxychlor alone. In the second study, the authors concluded that pretreatment with various dose levels of carbon tetrachloride inhibited the biliary excretion of methoxychlor metabolites in a dose-dependent manner. Although the data suggest a trend, there are not significant differences and, therefore, the conclusions are considered only tentative.

The major metabolites in feces and urine of female Swiss mice given ^{3}H-ring-labeled or ^{14}C-ring-labeled methoxychlor in a 4:1 mixture of olive oil and acetone as a single oral dose of 50 mg/kg bw were identified (by thin layer chromatography and compared with standards) as the monophenol

[2-(p-methoxyphenol)-2-(p-hydroxyphenyl)-1,1,1-trichloroethane] and bisphenol [2,2-bis-(p-hydroxyphenyl)1,1,1-trichloroethane] resulting from O-demethylation of methoxychlor, and as 2,2-bis(p-hydroxyphenyl)-1,1-dichloroethylene, the dehydrochlorination product of the bisphenol. Other metabolites present in significant quantities were bis(p-hydroxyphenyl)-acetic acid and p,p'-dihydroxybenzophenone. Methoxychlor itself was apparently not dehydrochlorinated because 2,2-bis(p-methoxyphenyl)-1,1-dichloroethylene was not detected (Kapoor et al., 1970).

In a subsequent study from the same laboratory (Coats et al., 1979) with the same dose regimen and animal strain, 7% of the radioactivity excreted in the urine and feces within 48 hours of dosing was associated with the parent compound, 30% with the monophenol and 23% with the bisphenol. Other metabolites were not quantified.

In vitro studies performed with hepatic microsomes from a variety of species indicate that methoxychlor is O-demethylated by the microsomal MFO system. Methoxychlor bound to hepatic microsomal cytochrome P-450 of rats, mice and rabbits, producing a Type I spectral change (i.e., a peak at 385 nm and a trough at 420 nm) in the optical difference spectrum (Kulkarni et al., 1975; Donovan et al., 1978). Compounds that produce this type of spectrum are usually substrates for the MFO system (Kulkarni et al., 1975).

Incubations of hepatic microsomes from rats or mice with radioactively labeled methoxychlor and an NADPH-generating system resulted in the production of the monophenol and bisphenol metabolites and in the evolution of formaldehyde (Kapoor et al., 1970; Bulger et al., 1978a; Coats et al., 1979;

Ousterhout et al., 1979). The evolution of formaldehyde was inhibited by hexobarbital and SKF-525A, indicating that the MFO system was involved in the O-demethylation of methoxychlor (Bulger et al., 1978a).

Methoxychlor was more extensively metabolized in vitro than was DDT. Coats et al. (1979) conducted parallel incubations of equimolar amounts of ^{3}H-ring-labeled p,p'-methoxychlor and p,p'-DDT with hepatic microsomes from female Swiss mice. At the end of the incubations, ~9.8% of the methoxychlor remained intact while 95% of the DDT remained intact.

The metabolism of methoxychlor appears on the whole to result in detoxification, as well as to render the compound more polar and thus subject to rapid excretion rather than storage. The monophenol and bisphenol metabolites, however, are thought to contribute to the estrogenic effects of methoxychlor (see Studies on the Estrogenic Mechanism of Action in Chapter VII).

On the basis of these in vivo and in vitro studies, a plausible pathway for the metabolism of methoxychlor is outlined in Figure III-1.

Excretion

In the previously described study by Kapoor et al. (1970), female Swiss mice excreted 98.3% of the administered radioactivity (50 mg ^{3}H-ring-labeled-methoxychlor/kg bw) in feces and urine within 24 hours. The ratio of radioactivity in urine to that in feces was 0.13. All of the administered dose was excreted in 11 days. No other comments on temporal concentration relationships or coefficients were made. By way of comparison, only

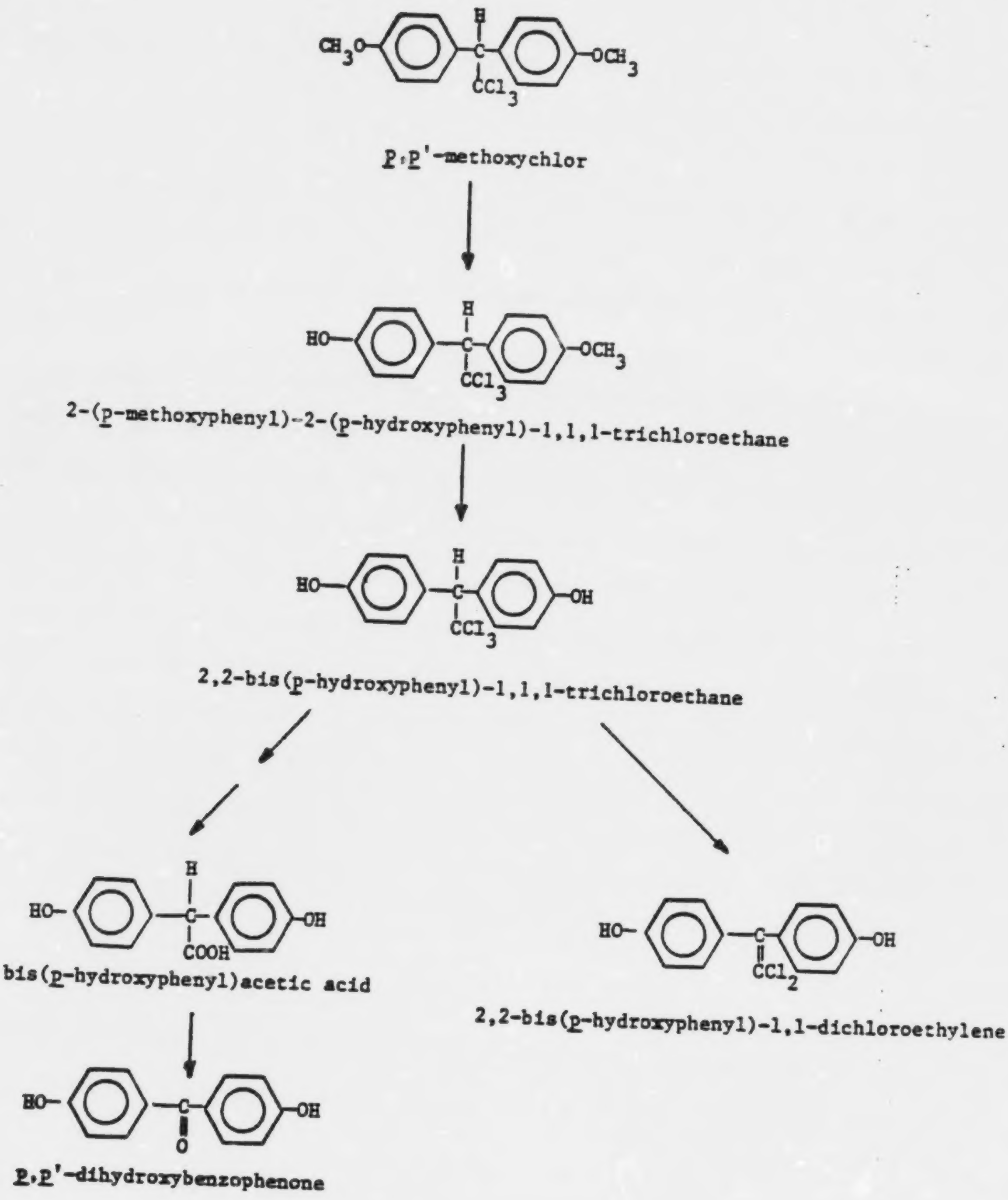

p,p'-methoxychlor
2-(p-methoxyphenyl)-2-(p-hydroxyphenyl)-1,1,1-trichloroethane
2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane
bis(p-hydroxyphenyl)acetic acid
2,2-bis(p-hydroxyphenyl)-1,1-dichloroethylene
p,p'-dihydroxybenzophenone

FIGURE III-1

Postulated Metabolic pathway for Methoxychlor in Rodents

Source: Kapoor et al., 1970

4.3% of the radioactivity from a smaller dose (12.5 mg/kg) of ^{14}C-DDT was excreted within 11 days.

Weikel (1957) studied the fate of ^{14}C-ring-labeled methoxychlor injected intravenously into adult male rats as a saline-lecithin-oil emulsion (3 mg methoxychlor/kg bw). The rapid disappearance of ^{14}C-methoxychlor from the blood and concomitant rapid accumulation of radioactivity in the liver was, according to the author, similar to that observed by others when particulate matter was injected intravenously; such particulates are taken up by the reticuloendothelial system. In intact rats, ~50% of the administered radioactivity was excreted in the feces, which was indicative of biliary excretion, and 5-10% was excreted in the urine within 4 days; the majority of this excretion occurred within the first 24 hours. In rats with cannulated bile ducts, ~20% of the administered dose was excreted in the bile during the first hour and ~50% was excreted in the bile within 6 hours, which confirms the initial findings. Urinary excretion of radioactivity by the cannulated animals during the first 6 hours was 0.1-0.2% of the administered dose, indicating that in the intact animals, much of the urinary excretion was derived after enterohepatic recycling.

Summary of Toxicokinetics

Pertinent data on the absorption, distribution, metabolism and excretion of methoxychlor by humans were not located in the available literature.

Quantitative data on absorption in laboratory animals is lacking; however, excretion of radioactivity in urine of mice administered ^{3}H-methoxychlor (Kapoor et al., 1970) indicates that some absorption from the gastrointestinal tract occurs. Systemic toxicity of methoxychlor following dermal exposure in laboratory animals indicates absorption by this route as well (Chapter V).

Rats (Adams et al., 1974; Villeneuve et al., 1972) and dogs (Hodge et al., 1952) did not accumulate or store methoxychlor in fat or other tissues to any great extent. Detectable levels of the insecticide were observed in the perirenal fat of rats fed relatively high dietary concentrations (≥100 ppm) for extended periods (≥4 weeks) (Kunze et al., 1950), but did not persist longer than 2 weeks after cessation of exposure. Similar results were obtained with very high dietary levels (1000-5000 ppm) (Harris et al., 1974).

The liver is the major site of metabolism of methoxychlor. Metabolites have been identified and a metabolic scheme based upon in vivo and in vitro studies in rats, mice and rabbits is presented (see Figure III-1). Metabolism results in detoxication and formation of rapidly excreted polar compounds.

Mice treated orally (Kapoor et al., 1970) and rats treated intravenously (Weikel, 1957) with radiolabeled methoxychlor excreted a greater percentage of the radiolabel in feces than in urine. Results in bile duct-cannulated rats indicate that an appreciable amount of biliary excretion and enterohepatic recycling occurred. Excretion by mice was complete in 11 days. The majority of the administered radioactivity was excreted in 24 hours by rats.

IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as methoxychlor from a variety of sources, including drinking water, food, ambient air, occupational settings and consumer products. This analysis of human exposure to methoxychlor is limited to drinking water, food and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works and travels, what one eats, and physiologic characteristics related to age, sex and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Detailed information concerning the occurrence of and exposure to methoxychlor in the environment is presented in another document entitled "Occurrence of Pesticides in Drinking Water, Food and Air" (Johnston et al., 1984). This chapter summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food and air.

In the Exposure Estimation section of this chapter, available information is presented on the range of human exposure and intake for methoxychlor from drinking water, food and ambient air for the 70 kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combined exposures from those three sources. However, the

Summary section of this chapter provides some insight into the range of intake values suggested by the available data.

Exposure Estimation

Drinking Water. Levels of methoxychlor in drinking water vary from one location to another. The highest level of methoxychlor monitored in the available studies was 50 μg/ℓ in Oregon (Elliott, 1979), below the Maximum Contaminant Level (MCL) of 100 μg/ℓ. However, in national studies, the highest level of methoxychlor reported was 0.09 μg/ℓ (U.S. EPA, 1984). Analysis of the National Screening Program for Organics in Drinking Water (NSP) (Boland, 1981) suggests that median levels of methoxychlor in drinking water systems would be below 0.1 μg/ℓ, since none of 116 systems sampled in the NSP contained a level of methoxychlor above 0.1 μg/ℓ. In addition, the median level of methoxychlor appears to be below 0.02 μg/ℓ since only 1 of 93 systems sampled in the Rural Water Survey (RWS) contained methoxychlor at a level above 0.02 μg/ℓ (U.S. EPA, 1984). Methoxychlor may not be present in drinking water in some areas. The available monitoring data are not sufficient to determine regional variations in exposure levels for methoxychlor.

The daily intake of methoxychlor from drinking water was estimated using the assumptions presented in Table IV-1 and the values presented above. The estimates in Table IV-1 indicate that the daily intake of methoxychlor from drinking water ranges from 0.0-1.4 μg/kg/day. However, the values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

TABLE IV-1

Estimated Intake of Methoxychlor from Drinking Water*

Drinking Water Concentration (μg/ℓ)	Intake (μg/kg/day)
0.0	0.0
0.02	0.0006
0.09	0.003
0.1	0.003
50	1.4

*Assumptions: 70 kg man consuming 2 ℓ of water/day

Diet. Data are limited on the dietary intake of methoxychlor in the United States. Daily food intake for adults and for infants and toddlers by food group was estimated by FDA for fiscal year 1979 (FDA, 1982a,b). The estimates of daily food intake are based on analyses of composite food samples obtained in market basket surveys. The daily intake of methoxychlor for adults for 1979, based on levels in dairy products, was 0.0032 µg/kg/day. The daily intakes for infants and toddlers for 1979 are presented in Table IV-2. Intake calculated on the basis of weight (µg/kg/day) is greatest for the infant and lowest for the adult. Dairy products were the major source of intake for all groups; fruit and fruit juices were a significant source of exposure for the toddler.

Additional data were obtained on the estimated total dietary intake of methoxychlor for adults in the years 1974-1979 and for infants and toddlers in the years 1975-1979 (FDA, 1980a,b, 1982a,b) (Table IV-3). The average total intakes for adults, infants and toddlers over the years studied was 0.0070, 0.0171 and 0.0083 µg/kg/day, respectively.

Using the above data, the daily intake of methoxychlor is estimated to be 0.0070 µg/kg/day. This value does not account for variances in individual exposure.

It is expected that dietary levels of methoxychlor vary somewhat with geographical location, with higher levels occurring in foods from areas near the sources of methoxychlor exposure. However, because of insufficient data, no estimates could be made of variations in intake by geographical region.

TABLE IV-2

Estimated Daily Intake of Methoxychlor by Food Class for 6-Month-Old Infant and 2-Year-Old Toddler[a]

Food Group[b]		Intake (μg/kg/day)[c]	
		Infant	Toddler
II.	Whole milk	0.0157	0.0063
III.	Other dairy and substitutes	--	0.0035
VIII.	Fruit and fruit juices	--	0.0049
	Total	0.0157	0.0147

[a] Source: FDA, 1982b

[b] Includes only those groups that contained quantifiable levels of methoxychlor.

[c] Values obtained by dividing intake in μg/day by 8.2 kg for infants and 13.7 kg for toddlers.

TABLE IV-3

Estimated Total Daily Intake of Methoxychlor for Adult Male, Infant and Toddler[a]

Year	Intake (μg/kg/day)		
	Adult Male	Infant[b] (6 months)	Toddler[b] (2 years)
1974	0.0114	--	--
1975	0.0037	Trace	ND
1976	0.0086	Trace	Trace
1977	0.0078	0.0066	0.0021
1978	0.0071	0.0289	0.0080
1979	0.0032	0.0157	0.0147
Average	0.0070	0.0171	0.0083

[a] Sources: FDA, 1980a,b, 1982a,b

[b] Trace residues were not included in calculating the average; nondetectable values assumed to equal zero.

ND = Not detected

Tolerance levels established by EPA for methoxychlor in and on raw agricultural commodities are presented in Table IV-4.

Air. No information was obtained on levels of methoxychlor in ambient air. Therefore, the intake of methoxychlor from ambient air could not be estimated.

Summary

Since no estimates could be made of the respiratory intake of methoxychlor in the United States, the relative contribution of the three sources (drinking water, food and air) to total intake could not be determined. However, based on estimates of drinking water and dietary intake presented in the Drinking Water and Diet Sections, the range of intake for the adult male for these two sources is estimated to vary from 0.0-1.4 μg/kg/day. This range is significantly lower than a maximum safe level of 20 μg/kg/day calculated by EPA (U.S. EPA, 1976).

TABLE IV-4

Tolerances for Methoxychlor In and On Raw Agricultural Commodities*

Commodity	Tolerance (μg/kg)
Alfalfa	100,000
Apples	14,000
Apricots	14,000
Asparagus	14,000
Barley, grain, stored (post-harvest)	2,000
Beans	14,000
Beets	
greens (alone)	14,000
with tops	14,000
without tops	14,000
Blackberries	14,000
Blueberries (huckleberries)	14,000
Boysenberries	14,000
Broccoli	14,000
Brussels sprouts	14,000
Cabbage	14,000
Carrots	14,000
Cattle, fat of meat	3,000
Cauliflower	14,000
Cherries	14,000
Clover	100,000
Collards	14,000
Corn	14,000
grain, stored (post-harvest)	2,000

TABLE IV-4 (cont.)

Commodity	Tolerance (μg/kg)
Cranberries	14,000
Cucumbers	14,000
Currants	14,000
Dewberries	14,000
Eggplant	14,000
Goats, fat of meat	3,000
Gooseberries	14,000
Grapes	14,000
Grasses, forage	100,000
Hogs, fat of meat	3,000
Horseradish	1,000
Horses, fat of meat	3,000
Kale	14,000
Kohlrabi	14,000
Lettuce	14,000
Loganberries	14,000
Melons	14,000
Milk, fat	1,250
Mushrooms	14,000
Nectarines	14,000
Oats, grain, stored (post-harvest)	2,000
Peaches	14,000

TABLE IV-4 (cont.)

Commodity	Tolerance (μg/kg)
Peanuts	14,000
forage	100,000
Pears	14,000
Peas	14,000
cowpeas	100,000
Peppers	14,000
Pineapples	14,000
Plums (fresh prunes)	14,000
Potatoes	1,000
Pumpkins	14,000
Quinces	14,000
Radishes	
tops	14,000
with tops	14,000
without tops	14,000
Raspberries	14,000
Rice, grain, stored (post-harvest)	2,000
Rutabagas	
tops	14,000
with tops	14,000
without tops	14,000
Rye, grain, stored (post-harvest)	2,000
Sheep, fat of meat	3,000
Sorghum, grain, stored (post-harvest)	2,000
Soybeans, forage	100,000
Spinach	14,000

TABLE IV-4 (cont.)

Commodity	Tolerance (µg/kg)
Squash	14,000
summer	14,000
Strawberries	14,000
Sweet potatoes (pre- and post-harvest)	7,000
Tomatoes	14,000
Turnips	
greens	14,000
with tops	14,000
without tops	14,000
Wheat, grain, stored (post-harvest)	2,000
Yams (pre- and post-harvest)	7,000
Youngberries	14,000

*Source: 40 CFR 180.120, July 1, 1981

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V. HEALTH EFFECTS IN EXPERIMENTAL ANIMALS

Effects of Acute Exposure

Technical grade methoxychlor contains ~88% p,p'-isomer; the remaining 12% contains o,p'-isomer plus contaminants of the commercial process (Metcalf, 1955). Lamoureux and Feil (1980) characterized 25 of the more than 50 impurities in technical methoxychlor. Although most of the information available on acute toxic exposures involves the use of the technical grade product, appropriate notations on the purity of methoxychlor have been included in the table on acute oral toxicity (Table V-1). Generally, the information available is on the acute toxicity of methoxychlor to experimental animals following the oral route of exposure.

Oral Exposure. When methoxychlor was mixed with a lipophilic vehicle for oral intubation studies there did not appear to be a great deal of variability in the acute oral LD_{50} doses within the same species. In studies with rodents, the strains used were unspecified (see Table V-1). The dose-response information in mice provided by von Oettingen and Sharpless (1946) does not seem to be incompatible with the LD_{50} dose reported by Domenjoz (1946a,b). The LD_{50} for mice reported by Coulston and Serrone (1969) appeared high, but it was the only value accompanied by standard error estimates and it was for the technical grade methoxychlor. Looking at the variability between species, it is possible to conclude that mice are more sensitive than rats to methoxychlor given in acute oral doses, but not enough information has been provided on sex, age, vehicle, strain, diet or general health and handling of the animals used to draw any definite conclusions. Most important, because confidence limits have not been given for any of this work, no difference in the susceptibility of certain species to methoxychlor can be identified.

TABLE V-1

Acute Oral Toxicity of Methoxychlor to Laboratory Animals

Species/Strain	Sex	No.	Weight/Age	Vehicle	Purity	Dose (g/kg bw)	Effects	Reference
Mice/NR	M	10	20-30 g	olive oil	purity unspecified, synthesized in investigators' laboratory	0.10	no deaths; no tremors observed at any dose	von Oettingen and Sharpless, 1946
		8				0.20	no deaths	
		10				0.30	no deaths	
		10				0.40	no deaths	
		8				0.50	no deaths	
		10				0.60	no deaths	
		10				0.70	no deaths	
		10				0.80	2/10 deaths	
		10				0.90	1/10 deaths	
Mice/NR	NR	NR	NR	olive oil	unspecified	1.85	LD_{50}	Domenjoz, 1946a,b
Mice/NR	NR	NR	NR	NR	technical grade	2.9 ± 0.5*	LD_{50}	Coulston and Serrone, 1969
Rats/Holtzman	M	7	200-250 g	corn oil	90% pure Grade II, Supplier: Sigma Chemical Co.	0.01	no effect on SGOT, SGPT, synthetase I, total synthetase or hepatic pyruvate; no hepatic pathologic changes observed at any dose	Morgan and Hickenbottom, 1979
		7				0.04	Significant decrease in hepatic lactate	
		7				0.16	Significant decreases in hepatic lactate and glycogen phosphorylase	
		7				0.64	Significant decreases in hepatic lactate and glycogen phosphorylase; significant increase in hepatic glucose-6-phosphatase; gradual dose-related decrease in hepatic glycogen levels but not significant. No histological changes in liver.	

TABLE V-1 (cont.)

Species/Strain	Sex	No.	Weight/Age	Vehicle	Purity	Dose (g/kg bw)	Effects	Reference
Rats/NR	F	35	200 g	corn oil	technical grade	5.0 (single or double dose)	LD_{50}; at 72 hours no hepatic or renal tissue pathologic lesions observed	Hodge et al., 1950
Rats/NR	M	35	275 g	corn oil	recrystallized; nearly 100% *p,p'*-isomer	5.82 (single or double dose)	7% mortality at 72 hours; 53% mortality at 120 hours; no hepatic or renal tissue pathologic lesions observed	
Rats/NR	NR	17 13	150-200 g	olive oil	unspecified; supplier: Dupont Chemical Co.	5.0 7.0	0% mortality 53.8% mortality; diarrhea, progressive weakness, and death in 36-48 hours; no neurologic effects observed	Smith et al., 1946
Rats/NR	NR	NR	NR	NR	purified	6.0	LD_{50}; depression of CNS occurring within 24 hours, lasting 2-4 days; tremors noted but not a prominent symptom	Lehman, 1951
Rats/NR	NR	NR	NR	NR	technical grade	>2.5	LD_{50}; 1 hour after a dose of 2.5 g/kg hepatic exam revealed enlarged intracytoplasmic vacuoles; at 24 hours, increase in rough ER, swollen mitochondria, small myelin droplets, induction of microsomal demethylase that persisted for 3 weeks	Coulston and Serrone, 1969
Rabbits/NR	NR	4	2.0-2.5 kg	intubation (olive oil)	unspecified; supplier: Dupont Chemical Co.	200 mg/kg bw/day (5-6 doses/week) up to 15 doses	100% mortality after 4-15 doses; diarrhea; anorexia; microscopic examination revealed fatty degeneration of the kidney with some epithelial necrosis; fatty degeneration of heart muscle and hepatic parenchyma, necrosis of gall bladder; pulmonary edema.	Smith et al., 1946; Lillie et al., 1947

TABLE V-1 (cont.)

Species/Strain	Sex	No.	Weight/Age	Vehicle	Purity	Dose (g/kg bw)	Effects	Reference
Hamsters/ Golden Syrian	M/F	NR	6 weeks	NR	unspecified	>2.0	LD_{50}; 14 days	Cabral et al., 1979
Monkeys/rhesus	NR	NR	NR	NR	technical grade	>2.5	LD_{50}; 24 hours after a dose of 2.5 g/kg hepatic microsomal demethylase activity induced that persisted for 3 weeks; swollen mitochondria and ER, increase in rough ER	Coulston and Serrone, 1969

*± standard error

NR = Not reported; M = male; F = female

Unlike exposures to many chlorinated hydrocarbon pesticides, acute methoxychlor exposures result in depression rather than stimulation of the CNS (Gosselin et al., 1976). In fact, in an experiment specifically designed to compare the oral toxicity and systemic effects of a series of DDT analogues and derivatives, Smith et al. (1946) observed none of the DDT-like CNS effects when methoxychlor was administered to rats (see Table V-1). Little detailed information is actually available on the clinical signs of acute methoxychlor poisoning. According to Gosselin et al. (1976), onset of symptoms is slower and recovery time is longer than what has been observed with DDT. Lehman (1951) states that depression of the CNS occurs within 24 hours and lasts 2-4 days in rats.

Hodge et al. (1950) observed that deaths of rats were delayed until 3 days after oral administration of LD_{50} doses of technical methoxychlor in corn oil. Recrystallized methoxychlor proved less toxic than the technical grade in that 50% mortality was not observed until the fifth day following such exposure (Table V-2) (females were used to test the technical product and males, the purified version). The cause of death was not documented. The technical material contained ~12% carbinol derivative and other methoxychlor isomers, while the purified compound was nearly 100% p,p'-isomer. No data were available on the impurities. In an acute feeding study of rats with an average initial body weight of 72 g, a diet containing 20% technical methoxychlor administered to one group of five males resulted in 100% mortality in <10 days (Hodge et al., 1950); a 5% methoxychlor diet resulted in 60% mortality (time of deaths unspecified) and growth retardation; a 1% diet caused marked growth reduction but no deaths, whereas a 0.1% diet caused no reduction in growth in 10 days when compared with controls.

TABLE V-2

Acute Oral Toxicity of Methoxychlor in Rats*

Methoxychlor	Dose (g/kg bw)	Number of Rats in Group	Cumulative Days (percent deaths)			
			1	2	3	5
Technical grade	2.87	10	0	0	10	NR
	4.62	10	0	0	40	NR
	5.95	15	0	0	53	NR
Purified	2.98	10	0	0	10	20
	4.07	10	0	0	10	20
	5.82	15	0	0	7	53

*Source: Adapted from Hodge et al., 1950

NR = Not reported

Histopathological examination revealed no hepatic damage, and organ weights demonstrated only those changes that could be attributed to refusal of food.

von Oettingen and Sharpless (1946) also observed that methoxychlor administered orally to mice did not produce the tremors characteristic of DDT intoxication. A single intravenous injection of methoxychlor in rats and rabbits, however, will produce signs of poisoning similar to those of DDT, and the dose necessary to produce these effects will be approximately the same (Winteringham and Barnes, 1955; Weikel, 1957). This suggests that the rate of detoxification of methoxychlor is considerably greater than the rate at which it is absorbed from the gastrointestinal tract. Laug and Kunze (1951) reported in an abstract that the oral administration of 0.5-1.0 mℓ carbon tetrachloride/kg given concurrently with methoxychlor (dose unspecified) to rats produced toxic symptoms similar to the characteristic tremors observed with DDT. The hepatic injury caused by the carbon tetrachloride presumably impaired the biotransformation of methoxychlor; the methoxychlor was now being absorbed at a faster rate than it could be detoxified (Winteringham and Barnes, 1955).

Results of pathological examinations following acute oral exposures of methoxychlor are available in some detail for rats and rabbits, while only sketchy information on monkeys has been located (see Table V-1). A study designed to elucidate the pharmacologic and pathologic actions of DDT and some of its analogs was published in two separate papers by Smith et al. (1946) and Lillie et al. (1947). This investigation was intended to establish, for the record, some comparisons of the similarities and differences in the toxicologic effects of these compounds. In general, the number of animals used was small, no mention of controls was made and the clinical and pathologic response of the animals to each compound was not discussed in detail. Lillie et al. (1947) administered single doses of 2-8 g methoxychlor/kg (purity and vehicle unspecified) to nine rats. Only one animal died, just 5 days after having received the lowest dose. Histopathological examination of this animal revealed many isolated hepatocytes in various stages of coagulative necrosis and fatty degeneration in the liver, kidneys and heart muscle. Two rats sacrificed on day 7 also exhibited hepatic necrosis and small granulomas (dose unspecified). Fatty degeneration of isolated hepatic and renal cells, focal interstitial nephritis, small foci of interstitial myocarditis, and pulmonary interstitial and perivascular lymphocytic infiltration were observed in the remaining animals.

Rabbits were sensitive to the oral administration (by gavage) of methoxychlor in olive oil (Smith et al., 1946). Exposure to 200 mg/kg/day (5-6 days/week) was lethal to 4/4 rabbits within 4-15 doses. Histopathologic examination revealed fatty degeneration of the kidney with some epithelial necrosis, fatty degeneration of the heart muscle and hepatic parenchyma, necrosis of the gall bladder and pulmonary edema. The most consistent alterations observed were the renal degenerative changes. All rabbits had either pneumonia or pulmonary edema, but these effects were attributed to accidental aspiration, secondary to gavage, by the authors.

Coulston and Serrone (1969) noted the induction of hepatic microsomal demethylase activity, which persisted for 3 weeks, and hepatic pathologic alterations following the oral administration of 2.5 g technical methoxychlor/kg to rats and monkeys (see Table V-1). An oral dose of 100 mg technical methoxychlor/kg/day for 4 days caused a distention in rat hepatic ER. Some spermatogenic arrest was observed in immature rats, but not in adult rats or monkeys. This observation could be of particular importance in light of the findings of Hodge et al. (1950) on arrested testis development in rats following subchronic feeding studies with methoxychlor (Effects of Subchronic Exposure Section). This publication (Coulstron and Serrone, 1969) is a review article on the role of nonhuman primates in the evaluation of drug toxicity in humans. The authors include information on a number of unrelated drugs in many species and the use of electron microscopy in the evaluation of the pathologic changes observed in numerous tissue specimens. Details of experimental design are not provided, however.

No hepatic pathologic changes were observed by Morgan and Hickenbottom (1979) 24 hours after single oral administrations of 0.01-0.64 g/kg of 90% pure methoxychlor to male Holtzman rats. Enzyme assays capable of detecting subtle biochemical changes in hepatic function (specifically glycogen metabolism) revealed changes in the liver (see Table V-1). When 0.1 g methoxychlor/kg (purity unspecified) was administered to male Holtzman and Wistar rats intraperitoneally, however, hepatic glycogen was increased maximally after 6 hours and remained elevated until 24 hours after injection (Hickenbottom, 1975). Glycogen phosphorylase and glycogen synthetase activities were both increased, with no changes in glucose-6-phosphatase in the Hickenbottom (1975) study. An explanation for these contradictory findings, apart from the different routes of administration, are not readily apparent.

Other Routes of Exposure. Little information is available on the toxicity of methoxychlor by routes other than oral. Lehman (1952a) stated that the approximate acute dermal LD_{50} (24 hours) in rabbits was >2.82 g/kg of a solution of 30% technical grade methoxychlor in dimethyl phthalate. Dermal absorption does occur, according to Lehman (1952a), with dermal application producing slight skin irritation but no symptoms of toxicity.

Methoxychlor proved to be more toxic than DDT when administered intravenously in rats (Lehman, 1954). The approximate LD_{50}s for methoxychlor (purity unspecified) and DDT were 29 mg/kg and 50-80 mg/kg, respectively. Signs of poisoning produced in rats and rabbits following an intravenous injection of methoxychlor were similar to those produced by DDT (WintWinteringham and Barnes, 1955), although the exact effects were unspecified by these authors.

In studies of behavioral and CNS responses in rats, methoxychlor (purity unspecified) alone and in combination with CNS-active drugs had no effect on spontaneous motor activity or convulsive seizure thresholds and did not alter the toxicity of amphetamine following 24-hour acute doses of one-half the LD_{50}, administered by an unspecified route (Weiss and Brodie, 1968).

Effects of Subchronic Exposure

Oral Exposure. Table V-3 summarizes the effects of subchronic oral administration of methoxychlor to laboratory animals. Growth retardation seems to be the most common feature of sublethal methoxychlor poisoning following ingestion (Lehman, 1952b). It should be noted, especially for rats, that refusal to eat as a result of an unpalatable taste would account for the observed weight loss. This effect has been seen repeatedly in studies with rats, dogs and swine before the manifestation of toxic effects on organ weights, hematology, blood chemistry or target organs. Stein (1970), however, reported that no significant disturbance in weight was observed in monkeys or men following oral adminstration of methoxychlor (Table V-4). Information contained in Stein's (1970) summary of studies on dogs, swine and humans seems to be identical to that reported by Tegeris et al. (1966); therefore, this latter study has not been summarized in Table V-4.

The article by Stein (1970) is a review of studies on methoxychlor; however, no references are provided. The source was a Pesticide Symposium held in 1968, but the article does not appear to be the minutes of such a meeting.

Species Differences -- Dogs and swine manifested distinctly different toxicity patterns following dietary administration of methoxychlor (1-4 g/kg bw/day) for periods of up to 10 months (Tegeris et al., 1966; Stein, 1970; Bierbower, 1966, 1967a,b). No behavioral disturbances were noted in swine, but dogs experienced tremors and convulsions. In fact, it was specifically noted that tremors were absent in rats fed for a full year on diets that

TABLE V-3

Subchronic Oral Toxicity of Methoxychlor

Species/Strain	Sex	Number	Weight/Age	Vehicle	Purity	Dose/Duration	Effects	Reference
Rats/ Sprague-Dawley	M	5-6/ group	37-41 g/NR	diet	technical grade	10,000 mg/kg diet x 33-55 days in pair-fed and nonpair-fed groups	Body weight loss, marked testicular atrophy, decreased weight of seminal vesicles, and ventral prostate glands, increase in adrenal weight, cystic tubular nephropathy in kidneys, histopathologic changes in testes and kidneys but not in liver, pancreas, or adrenal glands.	Tullner and Edgcomb, 1962
Rats/NR	M-F	20/group	average weight, 54 g/NR	diet	technical grade	100 mg/kg diet x 45 days	No effect on growth, survival, organ weight, hematology, or histopathologic examination of tissues.	Hodge et al., 1950
						1000 mg/kg diet x 45 days	Small but detectable growth depression, no effect on survival, organ weights, hematology, or histopathologic examination of tissues.	
						10,000 mg/kg diet x 30 days[a]	Marked decrease in testes weight (0.2 g vs. 1.8 g in controls) and development, histologic testicular atrophy, minor decreases in growth rate, red blood cell counts, and hemoglobin, no histopathologic changes in any organ except testes.	
						30,000 mg/kg diet x 45 days	Marked growth retardation, 8/10 deaths in each of the male and female groups, histologic changes in testes, (but not in liver, kidneys, spleen, heart, stomach, lungs or brain), dramatic decreases in organ weights; no effect on hematology.	

TABLE V-3 (cont.)

Species/Strain	Sex	Number	Weight/Age	Vehicle	Purity	Dose/Duration	Effects	Reference
Rats/ Sprague-Dawley	M	12	322 g/ 76-91 days	diet (mixed with aqueous starch)	technical grade	2000 mg/kg diet x 90 days	Marked growth depression, significantly decreased testicular and ventral prostate weights, (11% decrease in testes weight, 23% decrease in body weight, and 40% decrease in ventral prostate weight)	Shain et al., 1977
Rats/ Sprague-Dawley	NR	12/group	NR/23 days	diet pair-fed and non-pair fed (acetone used for mixing in diet)	sigma grade II	10 mg/kg diet x 16 weeks	Slight reduction (not statistically significant) in hepatic storage of Vitamin A (7-13%).	Davison and Cox, 1976
						100 mg/kg diet x 16 weeks	Decreased hepatic storage of Vitamin A.	
						1000 ppm x 16 weeks	Decreased body weight gain in nonpair-fed rats only.	
						10,000 mg/kg diet x 16 weeks	4/12 deaths; increase in liver weight relative to body weight	
Rats/NR	M	NR	NR/NR	diet	NR	500 and 2500 mg/kg diet x 4 weeks 500 and 2500 mg/kg diet x 52 weeks	Body weight loss, decreased food intake, increased hexobarbital sleeping time, elevated pentylenetetrazol and hexafluorodiethyl ether convulsive thresholds; no effect on survival, cardiovascular parameters, liver weight, histopathologic examination, or spontaneous motor activities.	Weiss et al., 1970
Rats/NR	M-F	14/group	(55-65 g)/ 30 days	diet	recrystallized in Pyrax	300, 600, 900 or 1200 mg/kg diet x 1 year	No effect on survival or growth; histopathologic examination performed on only 1 animal (600 ppm) in these 4 groups; no changes observed in heart, lungs, GI tract, liver, spleen, kidneys, adrenals, pancreas, thyroid, gonads, skeletal muscle or skin.	Haag et al., 1950
						2500 mg/kg diet x 1 year	Growth retardation; no histopathologic changes observed in the one rat examined from the group.	

TABLE V-3 (cont.)

Species/Strain	Sex	Number	Weight/Age	Vehicle	Purity	Dose/Duration	Effects	Reference
Rats/NR (cont.)						5000 mg/kg diet x 1 year	Growth retardation; no increase in mortality, hemosiderosis in spleen in one rat; no other histopathologic changes observed in the seven animals examined in this group.	
						10,000 mg/kg diet x 1 year	7/7 females and 2/7 males died within 10 weeks; emaciation and lack of growth; no tremors observed; hemosiderosis of spleen in one rat; no other histopathologic changes observed in 7 animals examined from this group (including brain).	
Dogs/NR	NR	2/group	NR	diet	technical grade	20, 100 and 300 mg/kg bw/day x 1 year	2 deaths, not treatment-related; no effect on growth, hematology, organ weights, urinalysis; no evidence of tissue damage during histopathologic examination of testes, liver, kidneys, spleen, heart, stomach, lungs, brain, large and small intestine, adrenals, bladder, lymph nodes and bone marrow.	Hodge et al., 1952
Dogs/beagle	M-F	6/group	5.4-12.8 kg/ 5-9 months	diet	technical grade	1.0 g/kg bw/day x 6 months	Loss of body weight; no effect on behavior, survival time, hematology or clinical chemistry measurements; no histopathologic tissue damage in thyroid, pancreas, adrenals, lungs, heart, liver, stomach, small intestine, large intestine, kidneys, bladder, prostate, testes, uterus, muscle, spleen, bone or mammary tissue.	Tegeris et al., 1966

TABLE V-3 (cont.)

Species/Strain	Sex	Number	Weight/Age	Vehicle	Purity	Dose/Duration	Effects	Reference
Dogs/beagle (cont.)		N = 8 in the 2.5 g/kg group				2.0, 2.5 or 4.0 g/kg/bw/day up to 6 months	Body weight loss; behavioral symptoms including salivation, tremors, mydriasis, hyperesthesia, tonic seizures, tetanic convulsions (survival time in these dogs averaged 22 days after onset of convulsions), ataxia; dose-dependent increase in SGOT, SGPT and alkaline phosphatase; dose-dependent absence of adipose tissue from normal depots and congestion of mucosa of small intestine; no effect on hematology; no histopathologic tissue damage.	
		4		intubation; 1% gum tragacanth		2.5 g/kg bw/day x 5 months	No signs or symptoms of toxicity (probably because of vehicle)	
Swine/ miniature	M-F	24 F 4 M	NR/6.5-23.5 months	diet	unspecified	1 g/kg bw/day for 6-40 weeks; (increased to 2 g/kg bw in 7 animals at 4-12 weeks)	2/28 deaths between 10 and 17 weeks; signs of continuous heat after 21 days, prolapsed vagina; swollen vulva, moderate to severe breast hyperplasia with secretion; pseudocyesis; endometrial hyperplasia in the uteri of all females; histopathological examination revealed chronic interstitial renal fibrosis, uterine cysts in all treated pigs, hypotrophic testes in 2/4 boars, moderate to severe fatty liver changes in 4 females, offspring had swollen vulvas and were in heat.	Bierbower, 1966
Swine/ miniature	M castrated	4	NR/mature	diet	unspecified	1-2 g/kg bw/day^{b} x 3.5 -5.5 months	Teats of all animals were maximally enlarged after 2.5 months (development of glandular tissue with secretory material in acini); chronic interstitial renal fibrosis.	Bierbower, 1967a
Swine/ miniature	M castrated	3	NR/11 weeks	diet	unspecified	2 g/kg bw/day x 18 weeks	Hyperplasia of the ducts in the mammary gland; (changes were less severe than with mature pigs) chronic interstitial renal fibrosis.	Bierbower, 1967b

TABLE V-3 (cont.)

Species/Strain	Sex	Number	Weight/Age	Vehicle	Purity	Dose/Duration	Effects	Reference
Swine/Hormel miniature	M-F	6/group	25-45 kg/ 8 months	diet	technical grade	1.0 or 2.0 g/kg bw/ day x 6 months or 4.0 g/kg bw/day x 4.5 months	Initial body weight loss in low- and mid-dose groups and then recovery, body weight loss seen throughout the study in high-dose group; in all groups; dose-dependent breast hyperplasia with milk secretion observed within 7 weeks, none came into estrus; excessively long hair on backs; dose-dependent elevation of blood urea nitrogen; histopathologic examination revealed advanced renal nephritis, (regardless of dose) hyperplastic and hypertrophic mammary glands, hypertrophic uteri, no effecton hematology, no histopathologic changes seen in liver, thyroid, pancreas, adrenals, lungs, heart, stomach, large and small intestine, bladder, prostate, testes, muscle, spleen or bone marrow.	Tegeris et al., 1966

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[a] This group was compared with a pair-fed group (i.e., rats who were given no methoxychlor in a food restricted diet, the same quantity of food consumed by treated rats. The pair-fed control showed similar weight reduction, indicating that this effect was due to reduced food consumption.

[b] Two animals were sacrificed after 3.5 months on a test diet of 1 g/kg/day; the 2 remaining animals were maintained on 1 g/kg diet for ~4.5 months and then given 2 g/kg for an additional 26 days before sacrifice.

NR - Not reported; M - male; F - female

TABLE V-4

Comparative Subchronic Oral Toxicity of Methoxychlor[a,b]

Species/Strain	Sex	Number	Weight/Age	Vehicle	Dose/Duration	Effects
Rats/ Sprague-Dawley	M-F	NR	NR	Intubation: 1% gum tragacanth	0.4, 1.0 or 2.5 g/kg bw/day x 3 months	Dose-related growth retardation in males and females after 4-6 weeks; salivation; alopecia at mid- and high doses; no effect on blood chemistry, no histopathologic tissue damage, no renal abnormalities; decrease in hepatic triglycerides.
Monkeys/rhesus	NR	NR	NR	Intubation: 1% gum tragacanth	0.4, 1.0 or 2.5 g/kg bw/day x 6 months	No effect on body weight or clinical chemistries; no histopathologic tissue damage; normal urinary estrogen pattern, no renal abnormalities; no change in activity of hepatic drug-metabolizing (demethylase activity) enzymes but persistent mitochondrial swelling noted in electron micrographs; decrease in hepatic triglycerides.
Humans	M	4/group	NR	Oral: 1% gum tragacanth	0.5, 1.0 or 2.0 mg/kg bw/day x 6-8 weeks	No effect on body weight or clinical chemistries; no abnormalities seen under microscopic examination in biopsies of small intestine, subcutaneous fat, bone marrow, testes or liver, no abnormalities in volume of ejaculate or sperm morphology; no renal abnormalities; electron-microscopy revealed no hepatic changes; no change in percent distribution of fatty acids.

[a] Source: Stein, 1970

[b] Purity of methoxychlor was unspecified.

NR = Not reported; M = male; F = female

contained as much as 10,000 ppm methoxychlor (Haag et al., 1950). The absence of tremors like those produced by DDT following ingestion of methoxychlor would be consistent with information on rodents discussed in the Effects of Acute Exposure Section. No renal abnormalities were observed in dogs, but chronic interstitial nephritis was consistently evident with all doses in male and female swine. Age of the test animals did not influence the development of chronic renal disease (Bierbower, 1967a). Increases in blood urea nitrogen (BUN) levels correlate with the renal pathology seen in swine. Marked dose-dependent breast hyperplasia with milk secretion and other endocrine disturbances were prevalent in all methoxychlor-treated pigs, but were not noted in dogs (Nonteratogenic Reproductive Effects Section) (Tegeris et al., 1966). Changes in the mammary glands of immature castrated male pigs were not as striking as those observed in mature castrated animals (Bierbower, 1967a,b). The survival time in dogs that had developed CNS disturbances was drastically affected. These animals lived, on the average, for only 22 days after symptoms developed. Behavioral abnormalities were not observed in monkeys (Stein, 1970).

Target Organ Toxicity --

Effects on the Liver. Moderate to severe fatty changes in the liver were seen in 4 of 24 female miniature swine (Bierbower, 1966), whereas Hodge et al. (1952) specifically mentioned the absence of hepatic damage following a 1-year feeding study that, however, used a much lower dose of methoxychlor in dogs. A dose-dependent increase in serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), usually indicative of hepatic injury, was reported in dogs by Tegeris et al. (1966) and Stein (1970), but microscopic tissue examination did not reveal any liver changes. An elevated alkaline phosphatase level represented neither histological or cytological obstruction to the flow of bile nor any pathological alteration associated with the kidneys, bones or parathyroid. The only adverse effects of oral methoxychlor administration observed in rhesus monkeys were liver-related changes (Stein, 1970). A persistent mitochondrial swelling was revealed by electron microscopy, but an induction of microsomal enzyme activity (demethylase activity) did not accompany this change. Coulston and Serrone (1969) reported that electron microscopy of liver tissue from rhesus monkeys (number unspecified) given 2500 mg technical methoxychlor/kg/day orally for 6 months revealed many distorted swollen mitochondria with a loss of cristae. Stein (1970) also noted a decrease in hepatic triglycerides in rats and monkeys.

Rats, on the other hand, demonstrated an increase in drug-metabolizing enzyme (unspecified) activity and smooth endoplasmic reticulum, but only when gavaged with the high dose of methoxychlor (2.5 g/kg bw/day, mixed with 1% gum tragacanth) and sacrificed in 24 hours; this increase could not be demonstrated after more than 1 week of continuous treatment (Stein, 1970). The decrease of hepatic triglycerides in rats and monkeys and the marked reduction of depot fat in dogs suggested a disturbance in neutral lipid transport according to Stein (1970). Cecil et al. (1974) documented some gender differences in rats (strain unspecified) in response to a 2-month feeding of a 100 ppm diet of technical methoxychlor (90% purity). Liver weight was decreased ($p<0.025$) and liver lipid content was increased ($p<0.025$) in the male rat but methoxychlor had no effect on the female. In an investigation of the effect of methoxychlor (diets containing 2500 or 5000 ppm for 6 weeks before mating and throughout pregnancy and lactation) on reproduction (Nonteratogenic Reproductive Effects Section), liver weights were increased but hepatic lipids were unaffected in female rats (Harris et al., 1974).

Effects on the Kidneys. Cystic tubular nephropathy was observed regularly in male weanling Sprague-Dawley rats fed diets that contained 1% technical methoxychlor for 1-2 months (Tullner and Edgcomb, 1962). No renal abnormalities were reported by Hodge et al. (1950) at this dose in rats or by Haag et al. (1950), but these investigators did not specify the strain of the animals used in their studies.

Renal histopathologic changes in rats have been reported by Tullner and Edgcomb (1962) and in swine by Bierbower (1966, 1967a,b), Stein (1970) and Tegeris et al. (1966). Abnormally small cystic tubules were observed in the kidneys of rats fed methoxychlor. Multiple cysts were largest in the area of the corticomedullary junction and were for the most part lined with flattened epithelial cells which were characteristic of the loop of Henle or the distal convoluted tubule (Tullner and Edgcomb, 1962). In swine, the capsule was focally thickened and scarred, as were wedges of the parenchyma; the residual tubules were strikingly dilated (Stein, 1970).

Various amounts of brownish pigment, characterized as lipofuscin, were observed in the cytoplasm of renal tubular epithelial cells (Bierbower, 1966). Fine granular brownish pigment was also observed in the cytoplasm of hepatic cells. A number of minor renal abnormalities were noted by Hodge et al. (1952) in dogs including pyelonephritis, chronic cystitis and renal granulomata observed in one control dog, in the two dogs fed the 20 mg/kg bw/day diet, and in the two dogs given the 300 mg/kg bw/day diet.

Effects on Reproductive System. Marked testicular atrophy (Non-Teratogenic Reproductive Effects Section) in rats as a result of prolonged dietary administration of technical methoxychlor has been documented by several investigators. This effect appeared, accompanied by decreased weights of the ventral prostate glands, at a dietary level of 2000 ppm technical methoxychlor administered for 3 months to Sprague-Dawley rats (Shain et al., 1977). A significant reduction of 23, 11 and 40% in body, testicular and ventral prostate weight, respectively, was reported. Both Hodge et al. (1950) and Tullner and Edgcomb (1962) had reported these effects earlier in rats but at much higher dietary levels of methoxychlor (see Table V-3). Russell et al. (1981) observed decreased weights of perfused testes and seminal vesicles and highly significant ($p<0.001$) increases in the number of degenerating spermatocytes in stage VII of spermatogenesis in a group of 8 male rats (250-300 g) maintained on a diet containing 50,000 ppm (5%) methoxychlor (unspecified purity) for 7 days, when compared with nontreated controls. Testicular effects from methoxychlor feeding were not investigated by Weiss et al. (1970) or Haag et al. (1950) in rats at these dose levels.

Histopathologic changes in testes, mammary glands and uteri, as a result of the estrogen-like effects of methoxychlor, have been documented by Tullner and Edgcomb (1962) and Hodge et al. (1950) in rats, and Tegeris et al. (1966), Stein (1970) and Bierbower (1966, 1967a,b) in miniature swine. Fewer spermatocytes were present in the seminiferous tubules of methoxychlor-treated rats than in the tubules of the pair-fed controls and no spermatozoa were found in the tubular lumina (Tullner and Edgcomb, 1962).

Atypical spermatocytes, some binucleate and others with pyknotic nuclei and sudanophilic cytoplasmic granules, were present in the tubules. Hodge et al. (1950) observed in pair-fed treated rats that seminiferous tubules were severely atrophied, contained no germ cells or were lined with immature germ cells, and that some primary spermatocytes exhibited varying degrees of necrosis. In acute feeding studies by Hodge et al. (1950) (see Effects of Acute Exposure Section), no testicular effect was observed in rats. The authors speculated that this discrepancy may be related to the age and weight of the rats when they were started on the test diets. Stein (1970) reported a similar observation that testicular hypoplasia was readily produced in rats only if their initial weight was <90 g. Tullner and Edgcomb (1962) felt that the testicular changes observed by them and by Hodge et al. (1950) could not have been related to the effects of reduced food intake on pituitary function because in both investigations spermatogenesis was normal in pair-fed control animals. Instead, it was suggested that these defects in gonadal development were the result of the suppressed production of the anterior pituitary gonadotropins by the estrogenic action of technical methoxychlor (Nonteratogenic Reproductive Effects Section, Chapter V and Studies on the Estrogenic Mechanism of Action Section, Chapter VII).

The degree of mammary gland hypertrophy and hyperplasia in miniature swine was comparable in the 1 and 2 g/kg groups and was most extensive in the 4 g/kg bw/day group (Tegeris et al., 1966; Stein, 1970). Teat enlargement was generally due to hyperplasia of the duct epithelium and was observed in most female pigs, in all mature castrated male pigs and, to a lesser degree, in immature castrated male pigs (Bierbower, 1966, 1967a,b). Endometrial hyperplasia was seen in the uteri of all females fed methoxychlor diets (Bierbower, 1966).

Effects on Central Nervous System. Clinical or behavioral manifestations of methoxychlor poisoning are rarely reported in studies with experimental animals. Tremors and convulsions were noted in dogs but not in swine or rats (Tegeris et al., 1966; Stein, 1970; Bierbower, 1966, 1967a,b; Haag et al., 1950). Studies on avoidance behavior in rats suggested a low level of behavior toxicity at high doses of methoxychlor (Weiss and Brodie, 1969). Oral administration of methoxychlor (purity unspecified) at one-eighth the LD_{50} (exact dose unspecified) for 30 days produced shifts in discriminated avoidance (lever pressing) that appeared to be related to the onset of toxic cumulative effects on the CNS.

Vehicle Effect -- There is a marked difference in the response of experimental animals to the oral administration of methoxychlor depending on the vehicle in which the methoxychlor is tested. No signs or symptoms of toxicity were observed when fasted beagle dogs were administered technical methoxychlor (2.5 g/kg bw/day x 5 months) suspended in 1% gum tragacanth by stomach tube (Tegeris et al., 1966). The same amount of methoxychlor, mixed in feed and given daily for 5 months, produced CNS effects and biochemical abnormalities (see Table V-3). Rats manifested some signs of toxicity when given, by gavage, the same concentration of methoxychlor in 1% gum tragacanth as used in dogs (Stein, 1970). Hodge et al. (1950), Tullner and Edgcomb (1962) and Davison and Cox (1976), however, had all previously reported their observations of markedly greater toxicity in rats at lower dietary concentrations of methoxychlor fed for shorter periods of time. Monkeys did not show any signs of poisoning when administered methoxychlor (up to 2.5 g/kg bw/day for 6 months) suspended in 1% gum tragacanth by stomach tube (Stein, 1970). No other information on the toxic effects of methoxychlor in monkeys was available for comparison. Methoxychlor is a moderately lipid soluble and (see Effects of Acute Exposure Section) was in all instances dissolved in a lipid vehicle. The most probable explanation for these discrepancies is malabsorption of methoxychlor in the gastrointestinal tract, which is due to the gum tragacanth. The difference in intake between dietary feed and gavage and the subsequent metabolism of methoxychlor, which could limit accumulation, would also help to account for such observations.

Dermal Exposures. Methoxychlor is more dangerous to handle when in solution than in a less absorbable, wettable powder or dust form. Lehman (1952a, 1954) stated that repeated daily dermal exposures of rabbits to 600 mg/kg methoxychlor (purity unspecified) would constitute an approximate LD_{50} dose. This observation was based on a 90-day exposure period. When 1200 mg/kg was administered, all rabbits died after 7-19 exposures. Estimated multiple dermal exposures of 36 g/day of methoxychlor would be dangerous to man, according to Lehman (1948). No documentation or details, other than those observations, are provided by the author.

Testicular atrophy, proportional to the dermally applied dose of methoxychlor, was observed in all surviving male rabbits, as reported by Nelson (1942) in an FDA memorandum (Table V-5). A summary of this memorandum (and others on methoxychlor) was made by Reuber (1980). The one surviving male rabbit in the high dose group developed severe chronic interstitial renal fibrosis, and one female given 30% methoxychlor in dimethyl phthalate in a volume of 1 mℓ/kg and 2 mℓ/kg/day, respectively, had

TABLE V-5

Subchronic Dermal Toxicity of Methoxychlor in Rabbits (strain, weight and age not reported)

Sex	Number	Vehicle	Purity	Dose/Duration	Effects	Reference
M-F	2M, 1F	30% methoxychlor in dimethyl phthalate	unspecified	0.5 mℓ/kg bw/day 5 days/week, 109 days	All rabbits developed severe anorexia, depression, and dose-dependent emaciation and hyperkeratosis; slight testicular atrophy; hyperplastic bone marrow in one male and one female.	Nelson, 1942
	2M, 1F			1.0 mℓ/kg bw/day 5 days/week, 109 days	Moderate testicular atrophy; focal nephritis (in one female); hyperplastic bone marrow.	
	3M			2.0 mℓ/kg bw/day 5 days/week, 109 days	1/3 males moribund and sacrificed on day 10; moderate atrophy of liver, spleen, bone marrow and pancreas; severe atrophy of testes; focal nephritis in 1 male; hyperplastic bone marrow.	
	2M, 1F			4.0 mℓ/kg bw/day 5 days/week, 109 days	1 male rabbit died on day 10, the female died on day 28, (necropsy in males showed: slight atrophy of liver, spleen, muscle, and pancreas; moderate atrophy of bone marrow; severe atrophy of testes; edema of wall of gall bladder) renal hypertrophy and chronic interstitial fibrosis in the surviving male; hepatic portal fibrosis and cellular infiltration; hepatic and testicular atrophy.	
NR	3/group	30% methoxychlor in dimethyl phthalate	unspecified	0.25, 0.5 mℓ/kg bw/day 5 days/week x 91 days	No effect on survival or growth; no abnormal histopatologic findings.	Haag et al., 1950
				1.0 mℓ/kg bw/day 5 days/week x 91 days	Moderate fatty degeneration of liver, disseminated nodules of cerebral cortex, perivascular infiltration, petechial hemorrhage of the brain.	
				2.0 mℓ/kg bw/day 5 days/week x 91 days	Growth retardation; diffuse fatty degeneration of liver in 2/3 rats, abnormal histopathologic findings in brain, sudden paralysis of foreleg.	
				3.0 mℓ/kg bw/day 5 days/week x 91 days	1/3 deaths in 1.4 weeks; growth retardation; diffuse fatty degeneration of liver and interstitial infiltration with lymphocytes; disseminated cortical nodules in brain; foreleg paralysis.	

NR = Not reported; M = male; F = female

focal nephritis. Haag et al. (1950), employing a very similar dosing schedule of methoxychlor (and DDT for comparison), did not report (but possibly did not look for) changes in the testes of their treated rabbits.

Histopathological findings were confined to the liver (diffuse fatty degeneration) and brain (small, sharply defined nodules) and were similar to those found with DDT (Nelson, 1942). The brain lesions were thought to be manifestations of a disease that is endemic in rabbits, known as encephalitis blastomatosis. Because the incidence of brain nodules was so high in this investigation, it was suggested that these two insecticides could be acting as predisposing factors. Slight or moderate atrophy was the only remarkable hepatic change noted by Nelson (1942).

Inhalation Exposures. No conclusions could be drawn from experiments conducted by Haag et al. (1950) in which dogs (2), rabbits (2) and rats (10) were exposed to atmospheres containing a dust of methoxychlor. Micronized dust containing 10% recrystallized methoxychlor was mixed with Pyrax (not described) plus 3% Santo-Cel (a dehydrated silica gel that increases the powder's flowability). The diluent of Pyrax plus Santo-Cel contributed to the toxicity of methoxychlor (and DDT for comparison) as judged by mortality and weight change figures. The authors stated that the differences in toxicity between DDT and methoxychlor dust were not pronounced between dogs and rabbits, but in rats methoxychlor was much less toxic than DDT. In a spray inhalation experiment, deodorized kerosene was used as the diluent for 2% recrystallized methoxychlor (Haag et al., 1950). Again, no conclusions could be drawn from the exposure of two dogs to this environment because the diluent killed the two control animals. Exposure of 10 rats to atmospheric concentrations of ~0.1 mg/ℓ of air, methoxychlor or DDT for 4 weeks (2 hours/day, 5 days/week) and their subsequent mortality ratios demonstrated that methoxychlor was more toxic than DDT to rats when inhaled in a kerosene spray.

Effects of Chronic Exposure

Table V-6 summarizes the chronic toxicity studies with methoxychlor. In most of the chronic studies reported in the literature, treatment with methoxychlor in the diet extended over a period of 18 months to 2 years. Levels up to a TWA concentration of 3491 ppm in male mice, a TWA of 1994 ppm in female mice (NCI, 1978), and 1600 ppm in male and female rats (Hodge et al., 1952; NCI, 1978) had no dose-related effects on mortality or longevity. A review by Reuber (1980) of a study made by Davis (1969) for the FDA (Table V-7) noted, however, that ingestion of 750 ppm technical grade methoxychlor in ground laboratory meal for up to 2 years in BALB/cJ male mice beginning at 3 weeks of age produced an average survival of 80.8 weeks in treated mice compared with 94.8 weeks in control mice. No satistical evaluation was given to indicate whether the treated groups differed significantly from controls.

Body weight gain was depressed by a TWA concentration of 997 ppm of technical grade methoxychlor in the diet of female mice (NCI, 1978) and by a TWA of 750-1000 ppm in female rats (Deichmann et al., 1967; NCI, 1978), but not at a level of 200 ppm methoxychlor in female rats (Hodge et al., 1952). Male mice ingesting a TWA concentration of 3491 ppm gained slightly less weight than controls during a 78-week treatment period, whereas no distinct retardation of growth was seen at a TWA of 1746 ppm methoxychlor (NCI,

TABLE V-6

.Chronic Toxicity of Methoxychlor

Species	Route	Dose/Duration	Effect	Reference
Rats, 50 male, Osborne-Mendel (for histopathology n = 19-50)	oral	360 mg/kg diet technical grade methoxychlor (95% purity) in corn oil for 29 weeks and then 500 mg/kg diet for 49 weeks^{a} (TWA 449 mg/kg diet)	Dose-related depression of body weight gain during treatment; no effect on mortality; no treatment-related histopathological effects in lung, liver, pancreas, kidney, or hematopoietic, endocrine, reproductive, musculoskeletal, circulatory or nervous systems	NCI, 1978
Rats, 50 male, Osborne-Mendel (for histopathology n = 18-50)	oral	720 mg/kg diet technical grade methoxychlor in corn oil for 29 weeks, then 1000 mg/kg diet for 29 weeks and then 1000 mg/kg diet cyclically^{b} for 29 weeks^{a} (TWA 845 mg/kg diet)	Same as above	NCI, 1978
Rats, 50 female, Osborne-Mendel (for histopathology n = 31-50)	oral	750 mg/kg diet technical grade methoxychlor in corn oil for 78 weeks^{a} (TWA 750 mg/kg diet)	Same as above	NCI, 1978
Rats, 50 female, Osborne-Mendel (for histopathology n = 30-50)	oral	1500 mg/kg diet technical grade methoxychlor in corn oil for 55 weeks and then 1500 mg/kg diet cyclically^{b} for 17 weeks^{a} (TWA 1385 mg/kg diet)	Same as above	NCI, 1978
Mice, male, B6C3F1, n = 50 (for histopathology n = 11-47)	oral	1400 mg/kg technical grade methoxychlor (95% purity) in corn oil in diet for 1 week and then 1750 mg/kg.diet for 77 weeks^{c} (TWA 1746 mg/kg diet)	No retardations of weight gains; no effect on mortality; no histopathological effect in lungs, liver, and hematopoietic, circulatory, and reproductive systems	NCI, 1978
Mice, male, B6C3F1, n = 50 (for histopathology n = 11-47)	oral	2800 mg/kg technical grade methoxychlor (95% purity) in corn oil in diet for 1 week and then 3500 mg/kg diet for 77 weeks^{c} (TWA 3491 mg/kg diet)	Slightly less body weight gain; no effect on mortality; no histopathological effect in lungs, liver, kidney, and hematopoietic, circulatory, and reproductive systems	NCI, 1978
Mice, female, B6C3F1, n = 50 (for histopathology n - 13-50)	oral	750 mg/kg technical grade methoxychlor (95% purity) in corn oil in diet for 1 week and then 1000 mg/kg diet for 77 weeks^{c} (TWA 1994 mg/kg diet)	Body weight depression; no effect on mortality; no histopathological effect in lungs, liver, kidney, and hematopoietic, circulatory, and reproductive systems	NCI, 1978

TABLE V-6 (cont.)

Species	Route	Dose/Duration	Effect	Reference
Mice, female, B6C3F1, n = 50 (for histopathology n - 13-50)	oral	1500 mg/kg technical grade methoxychlor (95% purity) in corn oil in diet for 1 week and then 2000 mg/kg diet for 77 weeks^c (TWA 997 mg/kg diet)	Body weight depression; no effect on mortality; no histopathological effect in lungs, liver, kidney, and hematopoietic, circulatory, and reproductive systems	NCI, 1978
Rats, Osborne-Mendel, n = 24	oral	0, 10, 25, 100, 500 and 2000 mg/kg diet technical methoxychlor in corn oil for 2 years	"mild stunting" of 2000 mg/kg diet group	Nelson and Fitzhugh, 1951
Rats, 30 male, 30 female, Osborne-Mendel	oral	80 mg/kg methoxychlor in diet for 2 years	No effect on longevity, mortality, food consumption, weight gain; primary pathology: chronic murine pneumonitis, testicular tumor in one male rat	Radomski et al., 1965
Weanling rats, 25 male, 25 female	oral	0, 25, 200, and 1600 mg/kg methoxychlor in diet for 2 years	Growth retardation at 1600 mg/kg; no dose related effect on survival time; normal values for urinary sugar and protein; normal red blood cell count, hemoglobin, white blood cell count, differential count; no histological changes in heart, lungs, spleen, stomach, intestines, adrenals, kidneys, testes, bladder, bone marrow, brain; no gross pathology or weight variation with dosage in liver, kidneys, lungs, heart, brain, spleen, stomach and testes	Hodge et al., 1952
Weanling rats, 30 male, 30 female, Osborne-Mendell	oral	0 and 1000 mg/kg recrystallized methoxychlor in diet for 27 months	Depression of body weight gain in female rats; normal hematocrit, hemoglobin, erythrocyte, and total and differential leucocyte counts; no effect of treatment on histology of heart, brain, pituitary, pancreas, stomach, small intestine, testes and ovaries; mean liver weight of females less than control, liver to body weight ratio unchanged; histopathology: hydropic swelling, granular cytoplasms, slight fatty metamorphosis, small focal areas of centrolobular necrosis, and passive congestions in liver; very slight infiltration of lymphocytes into renal cortex, and very slight congestion in tubules	Deichmann et al., 1967

TABLE V-6 (cont.)

Species	Route	Dose/Duration	Effect	Reference
Rats, 12 male, 12 females	oral	0, 10, 25, 100, 200, 500 and 2000 mg/kg diet for 2 years	Growth depression at 200 mg/kg diet and above in females	Lehman, 1965
Dogs	oral/ diet	1000, 2000, and 4000 mg/kg bw/day for 2 years	Loss of body weight; dose dependent increase of SGOT, SGPT and alkaline phosphatase; normal hematology; general loss of subcutaneous fat at necropsy; 2000 + 4000 mg/kg: behavioral change	Stein, 1968
Dogs, 1 male, 3 female	oral/ diet	300 mg/kg/day methoxychlor in capsules as 10% solutions in corn oil for 3.5 years	Increased fat in kidney of one dog; small number of hepatic cells with foamy cytoplasm in one dog that had a hepatocellucellular carcinoma; fibrotic areas containing macrophages with yellowish pigment in liver of second dog; small amount of fat in liver; increased lipid in adrenals; chronic cholecystitis in both dogs (early death at 105 and 260 days for male and one female dog)	Nelson and Radomski, 1953
Swine	oral/ diet	1000 and 2000 mg/kg bw/day for 2 years	Loss of body weight for first 8 weeks; then slow gain in weight	Stein, 1968
Swine	oral/ diet	4000 mg/kg bw/day for 2 years	Loss of body weight for first 8 weeks; original weight not regained	Stein, 1968
Swine	oral/ diet	1000, 2000 and 4000 mg/kg bw/day for 2 years	Increase of BUN; marked mammary hyperplasia with milk secretion; anestrus; growth of long black hair on back; chronic interstitial nephritis, increase of uterine weight	Stein, 1968

[a] 33-week observation period followed treatment

[b] Cycle: 4 weeks of treatment and 1 week dosage-free treatment

[c] 15-week observation period followed treatment

TABLE V-7

Survival Times for Male and Female Mice Ingesting Methoxychlor*

Dose (ppm)	Survival Time (weeks)			
	Males		Females	
	Average	Range	Average	Range
BALB/c Mice				
0	94.8	60-104	96.2	59-104
750	80.8	55-105	95.2	59-104
C3H Mice				
0	101.4	89-104	98.0	71-104
750	99.4	71-104	98.5	81-104

*Source: Adapted from Davis, 1969

1978). A concentration of 100 ppm recrystallized methoxychlor in the diet for 27 months in male rats had no effect on body weight gain (Deichmann et al., 1967), although a TWA concentration of 448 ppm of technical grade methoxychlor did depress body weight gain in male rats (NCI, 1978). As in female rats, no effect on weight gain was seen in males at levels of 200 ppm methoxychlor in the diet (Hodge et al., 1952).

Ingestion of technical grade methoxychlor (95% minimum purity) for a TWA concentration as high as 3491 ppm in male $B6C3F_1$ mice and a TWA of 1994 ppm in female mice of the same strain for 78 weeks (initial age: 6 weeks) produced no evidence of an effect of methoxychlor on physical appearance and behavior. The signs observed in both treated and control mice included sores on the body, a hunched appearance, localized alopecia, penile, vulvar or anal irritation, rough fur and bloating (NCI, 1978). Clinical signs observed in Osborne-Mendel rats of both sexes (initial age: 44 days) ingesting methoxychlor at TWA concentrations up to 845 ppm (males), and 1385 ppm (females) for 72-74 weeks and in control rats included hunched appearance, urine staining, body sores, rough fur, and cloudy, squinted or reddened eyes. These symptoms were seen slightly more frequently in the treated groups from the 34th week of treatment to cessation of treatment (NCI, 1978).

Lehman (1965) reported the results of a feeding study in rats (FDA, 1951) following administration of 0, 10, 25, 100, 200, 500 and 2000 mg/kg in diet of methoxychlor for 2 years. Growth retardation was observed at 200 ppm and above in animals. No histological damage attributable to the chemical was noted in animals over a 2-year period. A no-effect level of 100 ppm (5 mg/kg) was identified in this study.

In the studies of Hodge et al. (1952), weanling rats of both sexes (25/sex) were fed 0, 25, 200 or 1600 ppm methoxychlor in the diet over a 2-year period. Normal values for sugar and protein were found in the urine. Normal red blood cell counts, hemoglobin values, white blood cell counts and differential counts were found during sampling at monthly intervals during the first 3 months, bimonthly intervals for the balance of the first year, and at 3-month intervals during the second year of the study. Deichmann et al. (1967) also reported normal hematocrit, hemoglobin and erythrocyte and total and differential leucocyte values in weanling Osborne-Mendel strain rats of both sexes (30 of each sex) fed 1000 ppm recrystallized methoxychlor in the diet for 27 months.

In a synergism study of oral carcinogens, Radomski et al. (1965) administered 80 ppm each of methoxychlor, Aramite, DDT, or thiourea alone or 80 ppm each of methoxychlor, Aramite, DDT and thiourea as a mixture in the diet supplemented with 1% cod liver oil to Osborne-Mendel rats of both sexes for 24 months (see Table V-6); they found no effect of treatment on longevity, mortality, food consumption or weight gain from the compounds administered singly or as a mixture.

Target Organ Toxicity

Gross examination of liver, kidneys, lungs, heart, brain, spleen, stomach and testes of rats revealed little variation in organ weights with increasing dosage (Hodge et al., 1952). Lung weights, which were heavier than normal (7-8 g vs. 1.8-2.8 g in controls), were attributed by the authors to the presence of advanced pulmonary disease.

Deichmann et al. (1967) studied synergism among pesticides using one-half the dietary concentrations reported to be carcinogenic for the rat liver in the diet. In this study one group of 30 rats/sex were fed 1000 ppm of recrystallized methoxychlor in the diet (Carcinogenicity Section). Similar gross organ changes were noted in both methoxychlor-treated and untreated control rats in this study. Congestion, hemorrhage, pneumonitis and some abscesses were seen in the lungs. Occasional mottling of the spleen was noted, and the gastrointestinal tracts of a few rats were congested. Livers of some animals were slightly enlarged, congested, mottled, or showed fatty changes. Mean liver weights of treated female rats were less than those of controls, but the liver-to-body weight ratio was not significantly different from that of controls. Mean liver weights of the treated male rats were not different from untreated controls.

Evidence indicating liver damage in dogs ingesting 300 mg/kg bw/day methoxychlor for 3.5 years was reported by Reuber (1980) in a review of carcinogenicity studies including one done by Nelson and Radomski (1953) for the FDA. Nelson and Radomski (1953) concluded that methoxychlor has but little cumulative morphological effect in the dog; the liver and adrenals are the organs most consistently affected, and those in slight degree.

In the Hodge et al. (1952) study (see Table V-6) no chemically-induced histological changes were noted in rat heart, lungs, spleen, stomach, intestines, adrenals, kidneys, testes, bladder, bone marrow and brains at any dose. No mention was made, however, of histologic examination of the liver or ovary, organs which were of concern after treatment with methoxychlor (see Effects of Subchronic Exposure Section). The absence of testicular atrophy was specifically noted by the authors. Incidental findings unrelated to treatment included chronic pulmonary pathology and mild chronic interstitial nephritis. A frequent cause of death during the second year was cancer (Carcinogenicity Section). Accumulation of methoxychlor in body tissues was low (see Distribution Section, Chapter III).

Dosing with 1000 ppm methoxychlor in the Deichmann et al. (1967) study in rats produced hydropic swelling, granular cytoplasm, slight fatty metamorphosis, small focal areas of centrilobular necrosis, and passive congestion in the liver. In the kidneys, there was very slight infiltration of lymphocytes into the cortex and very slight congestion in the tubules. Moderate to marked congestion in the red pulp with slight hemolysis, megakaryocytes and an increase in hemosiderin-filled macrophages were observed in the spleen. In the lungs, moderate lymphocytic cuffs and follicles above blood vessels with scanty mitosis were observed. Similar histological changes were found, however, in the spleen and lungs of control rats.

At all dose levels (1000, 2000, 4000 ppm in the diet) in pigs, chronic interstitial nephritis was noted (Stein, 1968). Similarly, mammary hyperplasia was found in pigs after chronic exposure to methoxychlor in the diet. Increase of uterine weight and anestrus was observed. Of the laboratory mammals tested in these few chronic studies, estrogenic effects found in shorter-term studies are apparent only in the pig.

These chronic as well as relevant subchronic studies are also summarized in the Interim Ambient Water Quality Criterion Statement for Methoxychlor (U.S. EPA, 1982).

Synergistic and Antagonistic Effects

Limited information was available on the effects of other factors on methoxychlor toxicity.

The oral co-administration of 0.5-1.0 mℓ carbon tetrachloride/kg with an unspecified dose of methoxychlor in rats resulted in tremors characteristic of DDT poisoning (Laug and Kunze, 1951). It was suggested that carbon tetrachloride impaired the biotransformation of methoxychlor by causing hepatic injury: methoxychlor was being absorbed faster than it was being detoxified (Winteringham and Barnes, 1955) (see Oral Exposure Section).

In behavioral and CNS response studies in rats, methoxychlor (purity unspecified) alone or in combination with chlorpromazine, a CNS-active drug, had no effect on spontaneous motor activity or convulsive seizure thresholds and did not alter the toxicity of amphetamine following 24-hour acute doses of one-half the LD_{50}, administered by an unspecified route (Weiss and Brodie, 1968).

Administration of 50 or 80 ppm each of methoxychlor, Aramite, DDT, or thiourea alone or 50 or 80 ppm each of the compounds in mixture in the diet to groups of 30 or 50 Osborne-Mendel rats of each sex for 2 years produced no treatment-related effects on longevity, mortality, food consumption, weight gain or tumor incidence (Radomski et al., 1965). Similar results were obtained by Deichmann et al. (1967) with these compounds administered in combinations of one-half the dietary concentrations of each reported to be tumorigenic for the rat (see Dermal Exposures and Carcinogenicity Studies in Rats Sections).

Carcinogenicity

Methoxychlor has been tested for carcinogenicity in a number of studies using rodents (Nelson and Fitzhugh, 1951; Hodge et al., 1952, 1966; Radomski et al., 1965; Davis, 1969; Deichmann et al., 1967; NCI, 1978) and other animal species (Nelson and Radomski, 1953; Bierbower, 1966, 1967a,b). Statistically significant increases in tumor incidences were not reported in any of these studies by the original authors. Reuber (1978, 1979a,b, 1980), however, after his reevaluation of histological materials from several of these studies, asserts that methoxychlor is carcinogenic for animals. NCI (1978) and IARC (1979) had determined that methoxychlor was not carcinogenic in animals. After a review of the available animal pathology data, the EPA Carcinogen Assessment Group has also concluded that methoxychlor has not been shown to be carcinogenic in the laboratory animal species tested.

Carcinogenicity Studies in Rats. Nelson and Fitzhugh (1951) allowed Osborne-Mendel rats to ingest technical grade methoxychlor at 0, 25, 100, 200, 500 or 2000 mg/kg in the diet for a period of 2 years. Each group of animals consisted of 12 males and 12 females, and at termination, animals were examined for gross lesions. Histological preparations were made from the gross lesions encountered at autopsy. One animal of the 100 mg/kg group had hyperplastic foci in the liver, while at the 500 mg/kg level, one liver nodule was observed, and at the 2000 mg/kg level, four rats had developed nonmalignant hepatic cell adenomas. No other changes were noted in other organs, and no malignant changes were observed.

In a second early chronic toxicity study, Hodge et al. (1952) fed 25 male and 25 female rats diets containing 0, 25, 200 or 1600 mg/kg of technical grade methoxychlor. In the first year of this study, 4-30% of each group of animals died as a result of infections that were only partially controlled. At the end of 2 years, the surviving animals were killed and examined for gross findings. The investigators prepared histological sections of the heart, lungs, spleen, stomach, small intestine, large intestine, adrenal, kidney, testes, bladder, bone marrow, brain and any obvious lesions. It should be noted that the liver was not routinely examined histologically. In female rats of the 1600 mg/kg group, there was a greater number of tumors of all organs as compared with control animals. The authors considered this increase to be coincidental because all of the tumors were types common to this species of rat and because there was no increase in tumors of any particular organ.

In a series of experiments the possibility of synergism in the tumorigenic potential of thiourea, Aramite [1-(p-tert-butylphenoxy)isopropyl-2-chloroethyl sulfite], DDT and methoxychlor was examined (Radomski et al., 1965; Deichmann et al., 1967). For this series of three experiments, the tumorigenic dose of each compound was determined from the literature. The tumorigenic dose was defined as the lowest dose of a compound which would produce tumors. It was considered that tumor induction was additive, and hence two compounds administered together, each at half of the lowest dose that had been shown to produce tumors, would be effective in producing tumors. For methoxychlor, the study of Nelson and Fitzhugh (1951) was used. The experimental diets were made in such a manner as to contain 78, 124 and 200% of the tumorigenic dose if the action of the compounds were solely additive. In the last two experiments, a group treated with methoxychlor alone was also included.

In the first experiment (Radomski et al., 1965), a group of 50 male and 50 female Osborne-Mendel rats were fed diets containing 50 mg/kg each of thiourea, Aramite, DDT and methoxychlor (78% of a tumorigenic dose) for 2 years. Rats that were moribund or sacrificed at termination of the study were examined for tumors, with particular attention paid to the liver because all four compounds were believed to produce liver tumors in rats. There was a nonsignificant increase in the incidence of malignant tumors in both males and females of the experimental group as compared with the control animals (26/99 rats and 15/100 rats with malignant tumors, respectively). In the second 2-year study using 30 male and 30 female rats in each group (Radomski et al., 1965), the dose of each insecticide was increased to 80 mg/kg (124% of the tumorigenic dose), and one group of animals was given a diet containing methoxychlor (80 mg/kg) alone. The total tumor incidence was unchanged in the two experimental groups as compared with the control. Only one liver tumor was observed in this study and this tumor was in one of the control female rats.

In the third experiment (Deichmann et al., 1967) 30 rats/sex/group (with same procedure as described previously), were exposed to 200% of the tumorigenic dose. In this study, each compound was incorporated into the diet at 50% of its respective tumorigenic dose. Two combinations of pesticides were used in the diet: the first consisted of 200 mg/kg Aramite, 200 mg/kg DDT, 1000 mg/kg methoxychlor (recrystallized) and 50 mg/kg thiourea. In the second combination, aldrin at 5 mg/kg was substituted for 50 mg/kg of thiourea. One group of rats was given a diet containing 1000 mg/kg of methoxychlor alone. Again there was no change, except that animals receiving the second mixture of pesticides showed a slight decrease in the tumor incidence among the three experimental groups and the control group. Liver tumors were rare; one tumor was observed in a rat on the diet containing Aramite alone and one in a rat recieving the first diet. In this series of experiments, methoxychlor alone or in combination with other pesticides failed to increase the incidence of tumors in the Osborne-Mendel rat. It should be remembered, however, that the dose of each individual pesticide was no greater than 50% of the lowest dose that previously had been shown to produce an increase in liver tumors in rats.

The NCI bioassay of methoxychlor (NCI, 1978) tested groups of 50 male and 50 female Osborne-Mendel rats and $B6C3F_1$ mice; control groups consisted of 20 male and 20 female Osborne-Mendel rats and $B6C3F_1$ mice. Rats were exposed to technical grade methoxychlor (95% pure) in the diet for 78 weeks, followed by a 33-week observation period without exposure to the test compound. The concentration of methoxychlor was adjusted during the study as indicated in Table V-8. The TWA concentration for the high and low dose groups, respectively, was 845 and 448 mg/kg for the male rats and 1385 and 750 mg/kg for the female rats. Necropsy was performed on all animals that died spontaneously or were killed when moribund or at the termination of the study. Histological examinations were performed on major organs and any gross lesions of all animals, except where cannibalism or autolysis precluded such studies.

The effects of this chronic exposure to methoxychlor were manifested as only mild changes in the general health of the rats. There was a dose-related weight loss in both male and female rats, and in the latter part of the exposure period (34-78 weeks) there was a slight increase in the treatment groups of the frequency of clinical signs of an unthrifty condition.

TABLE V-8

Design Summary For NCI Bioassay of Methoxychlor in Osborne-Mendel Rats[a]

Sex	Initial Group Size	Methoxychlor Concentration (mg/kg in the diet)	Observation Period		Time-Weighted Average Concentration[b]
			Treated (weeks)	Untreated (weeks)	
		MALE			
Control	20	0		111	0
Low Dose	50	360	29		448
		500	49		
		0		33	
High Dose	50	720	29		845
		1000	29		
		1000[c]	16	4	
		0		33	
		FEMALE			
Control	20	0		111	0
Low Dose	50	750	78		750
		0		33	
High Dose	50	1500	55		1385
		1500[c]	17	6	
		0		34	

[a] Source: Adapted from NCI, 1978

[b] Time-weighted average concentration = $\frac{\Sigma(\text{concentration x weeks received})}{\Sigma(\text{weeks receiving treatment})}$

[c] These concentrations were cyclically administered with a pattern of one dosage-free week followed by 4 weeks of treatment at the level indicated

The only tumors observed at a higher incidence were hemangiosarcomas (Table V-9). Although historically this tumor type was not frequently observed in this strain of rats, the authors concluded that the increase was not a good indicator of the carcinogenicity of methoxychlor because the response was neither dose-related nor statistically different from control values. Other tumors in the treated animals also occurred in the controls at the same frequency. It was concluded that under this experimental regimen, methoxychlor was not tumorigenic to Osborne-Mendel rats.

Carcinogenicity Studies in Mice. Mice of strain $B6C3F_1$ were used in an NCI chronic toxicity assay of methoxychlor (NCI, 1978). Fifty males and 50 females were exposed to methoxychlor in the diet at two dose levels (Table V-10). The control group consisted of 20 mice of each sex. The dose levels were increased 1 week after the start of treatment; the TWA methoxychlor concentration for the high and low dose groups, respectively, was 3491 and 1746 mg/kg for the male mice and 1994 and 997 mg/kg for the female mice. The animals were maintained on the test diet for 78 weeks, and an additional 15 weeks were allowed to elapse before the termination of the study. The animals were examined for both gross and histologic pathologic alterations at the time of spontaneous death or when killed when moribund or at termination of the study.

Following chronic exposure to methoxychlor, there was a decrease in body weight gain in both treatment groups of female mice and in the high dose group of male mice. Other gross pathologic changes were normal for aging mice and appeared in both the control and treated animals. Both gross and histopathologic findings revealed a variety of tumors in this study, but the

TABLE V-9

The Incidence of Hemangiosarcomas in Rats Fed a Diet Containing Methoxychlor*

Site of Hemangiosarcomas	Male Rats			Female Rats		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Spleen	1/20	6/44	2/42	1/20	NR	NR
Subcutaneous	NR	2/50	NR	NR	NR	NR
Abdominal tissue	NR	1/50	NR	NR	NR	NR
All sites	1/20	9/50	2/50	1/20	NR	NR

*Source: Adapted from NCI, 1978

NR = Not reported

TABLE V-10

Design Summary For NCI Bioassay of Methoxychlor in B6C3F$_1$ Mice[a]

Group	Initial Group Size	Methoxychlor Concentration (mg/kg in the diet)	Observation Period		Time-Weighted Average Concentration[b]
			Treated (weeks)	Untreated (weeks)	
		MALE			
Control	20	0		92	
Low Dose	50	1400	1		
		1750	77		1746
		0		14	
High Dose	50	2800	1		
		3500	77		3491
		0		15	
		FEMALE			
Control	20	0		92	0
Low Dose	50	750	1		
		1000	77		997
		0		15	
High Dose	50	1500	1		
		2000	77		1994
		0		15	

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[a] Source: Adapted from NCI, 1978

[b] Time-weighted average concentration = $\frac{\Sigma(\text{concentration x weeks received})}{\Sigma(\text{weeks receiving treatment})}$

[c] These concentrations were cyclically administered with a pattern of one dosage-free week followed by 4 weeks of treatment at the level indicated

types and incidences of these tumors were similar, as indicated by statistical analysis, in both the control and experimental animals. It was concluded that under the conditions of this study, methoxychlor was not carcinogenic to $B6C3F_1$ mice of either sex.

Hodge et al. (1966) used methoxychlor in a validation of skin painting and s.c. injection as routes of exposure for the evaluation of a compound's carcinogenicity in mice. For the skin painting study, 50 male and 50 female C3H/Anf mice (2-4 months of age) were used in each group, and methoxychlor was applied to the shaved skin in acetone (0.1 or 10.0 mg of methoxychlor per mouse) once each week for the life of the animal. The treatment produced neither gross nor histological changes (histology was performed on 28 low dose animals and 23 high dose animals) in the skin. The results of the gross autopsy examination also revealed no changes in treated animals as compared with control animals. Similar negative findings were reported for the 50 mice of each sex receiving 10 mg/mouse of methoxychlor as a single s.c. injection. The compounds Aramite, Flectol H (1,2-dihydro-2,2,4-trimethyl-quinoline), and aminotriazole, which had been reported in the literature to be carcinogenic when incorporated into the diet of rats, were also negative in this assay. The authors concluded that these two assay procedures may be inappropriate for the detection of weak carcinogens.

Discussion. Reuber (1978, 1979a,b, 1980) reviewed the carcinogenicity studies of methoxychlor in rats and mice discussed above. Reuber (1980) considered the rat studies of Hodge et al. (1952), Deichmann et al. (1967) and Radomski et al. (1965) to be suggestive of a carcinogenic effect of methoxychlor when total tumor incidence in treated rats was compared to that in control animals. Reuber also considered the study of Radomski et al. (1965) to be evidence that methoxychlor was a hepatocarcinogen; however, in the statistical analysis from which his conclusion was drawn, a control incidence of 1 liver tumor in 50 rats was used instead of the value of 4 liver tumors in 50 rats as reported by Radomski et al. (1965). The early mouse study of Hodge et al. (1966) was considered to be inadequate for the evaluation of the carcinogenicity of methoxychlor because skin painting and s.c. injection were used as routes of administration instead of the oral route.

Reuber also examined histologic specimens from several of the carcinogenicity studies. A great deal of dissimilarity exists between the interpretation of the histologic specimens by the original investigators for NCI (1978) and the interpretation by Reuber (1978, 1980). Reuber (1978, 1980) concluded that the histologic evidence showed methoxychlor to be carcinogenic, causing an increase in liver and ovary tumors in female rats, and an increase in hemangiosarcomas of the vertebrae and total malignant neoplasms in female mice. Similarly, on reevaluation of the Nelson and Fitzhugh (1951) study with rats, Reuber (1979a, 1980) observed an increased incidence of liver and ovary tumors in females and liver tumors in males. The observed increase in ovarian tumors occurred only in rats ingesting diets containing 500 mg/kg of methoxychlor and not in the high dose group (2000 mg/kg). In the statistical evaluation of the incidence of liver tumors, the incidence of tumors in the control rats was obtained from a colony of rats of the same strain maintained by Reuber (1980) and not the control animals used in the Nelson and Fitzhugh (1951) study. The differences in the interpretation of the histologic specimens and in the conclusions drawn by Reuber (1978, 1979a,b, 1980) and the original investigators may in part be related to the difficulty in distinguishing between regenerative hyperplasia, hyperplastic nodules, benign neoplasia and malignant neoplasia, and in the use of inappropriate control data for the statistical analysis.

Reuber (1979b, 1980) also reviewed two chronic toxicity assays of methoxychlor that were reported in memoranda to the FDA (Nelson and Radomski, 1953; Davis, 1969). In the Nelson and Radomski (1953) study, four dogs (1 male and 3 female) were fed methoxychlor at a dose of 300 mg/kg/day for a maximum of 3.5 years. Early deaths at 105 and 260 days occurred for the male and one female dog. Reuber's reexamination of the histological specimens of two female dogs that survived the 3.5 year exposure indicated that one female dog had developed a liver carcinoma and the other developed fibrosis of the liver. Nelson and Radomski (1953) did not note the development of the carcinoma. As a result of the small number of animals used in this study, any interpretation of these findings would be problematic. In the Davis (1969) study, 100 male and 100 female mice of two strains (BALB/c and C3H) were fed diets containing 750 ppm of methoxychlor for 2 years. Liver tumors were observed in male and female BALB/c mice and in male C3H mice, and carcinomas of the testes were observed in male BALB/c mice (Table V-11). Malignant tumors at all sites were also increased in BALB/c mice of both sexes. Davis (1969) stated that a preliminary survey of these data showed that methoxychlor was not carcinogenic. He also stated, however, the need for a complete statistical analysis.

TABLE V-11

Tumor Incidence in Mice Ingesting Methoxychlor in the Diet[a,b]

Dose of Animals (ppm)	Strain	Sex	Site of Tumor	Incidence	Percent with Tumors
0	BALB/c	M	testis	8/71	11
750	BALB/c	M	testis	27/51	53
0	BALB/c	M	liver	3/73	4
750	BALB/c	M	liver	18/67	27
0	BALB/c	F	liver	2/89	2
750	BALB/c	F	liver	12/96	13
0	C3H	M	liver	23/96	24
750	C3H	M	liver	43/92	47
0	C3H	F	liver	11/99	11
750	C3H	F	liver	15/83	18

[a] Source: Adapted from Reuber, 1980

[b] A reevaluation of the FDA study

Conclusion. Methoxychlor has been assayed in a number of chronic exposure studies to determine whether or not it is a carcinogen. These studies have been performed on mice and rats by exposure to the compound in the diet. In addition, two limited studies on mice have been performed using s.c. administration and skin application of methoxychlor. There is some disagreement regarding the interpretation of these results; however, in a review of methoxychlor, the IARC (1979) concluded that the evidence did not support the contention that methoxychlor was a carcinogen. A similar conclusion was reached from the NCI (1978) bioassay of methoxychlor in rats and mice. In addition, the EPA Carcinogen Assessment Group has also concluded that methoxychlor has not been shown to be carcinogenic in the laboratory animal species tested. Two recent reviews of the NCI bioassay program, however, indicate that: 1) the bioassays are not designed to detect low-potency carcinogens, and 2) the evidence concerning carcinogenicity of methoxychlor was felt to be inconclusive, not negative (Griesemer and Cueto, 1980; Harper et al., 1982).

Mutagenicity

Technical grade methoxychlor has been assayed in a number of *in vitro* microbiological systems for mutagenic activity. Ashwood-Smith et al. (1972) used the spot assay and measured the reverse mutation of a tryptophan auxotroph of *Escherichia coli* WP2 in the absence of a metabolic activation system. Probst et al. (1981) tested methoxychlor for mutagenicity in eight *Salmonella typhimurium* histidine auxotroph strains (G46, TA1535, TA1000, C3076, TA1537, D3052, TA1538 and TA98) and two *E. coli* tryptophan auxotroph strains (WP2 and WP2 UVR A^-) in a 10,000-fold concentration range with and without metabolic activation by Aroclor-induced rat liver S-9 fraction enzymes. Simmon (1979) and Waters et al. (1982) also reported results of assays for methoxychlor mutagenic activity in the Ames S. typhimurium system with strains TA1535, TA1537, TA1538, TA100 and TA98, the E. coli WP2 tryptophan auxotroph assay, and the Saccharomyces cerevisiae D3 mitotic recombination assay both with and without a metabolic activation system from Aroclor-pretreated rats. Assays of methoxychlor-induced DNA damage were also performed on repair-proficient and repair-deficient strains of E. coli (strains W3110 and P3478, respectively) and strains of Bacillus subtilis (strains H17 and M45, respectively). Methoxychlor was determined to be negative in all of the above assays.

Technical grade methoxychlor was also negative in unscheduled DNA synthesis assays in primary rat hepatocyte cultures (Probst et al., 1981) and cultured human fibroblast W138 cells (Simmon, 1979; Waters et al., 1982) and in the sex-linked recessive lethal assay in Drosophila melanogaster (Waters et al., 1982; Benes and Sram, 1969). In D. melanogaster, methoxychlor was highly toxic; the largest dose tolerated was 0.2 μℓ injection of a 0.1% solution (Benes and Sram, 1969). Methoxychlor was negative at doses of 0.05-50 μg/mℓ in transformation assays using Syrian hamster embryo cells (Pienta, 1980). Dunkel et al. (1981) also reported negative results for transformation response in Syrian hamster embryo cells at methoxychlor doses up to 50 μg/mℓ as well as in Rauscher murine leukemia virus-infected rat embryo cells taken through eleven passages at methoxychlor doses up to 12 μg/mℓ. Traul et al. (1981) also reported a negative response of methoxychlor in the latter systems.

A positive dose-related response was observed for cell transformation in BALB/3T3 cells at methoxychlor doses up to 2.0 μg/mℓ: a 3-fold increase in the number of foci/plate above control (untreated) cells was observed (Dunkel et al., 1981).

Technical grade methoxychlor has been reported to be ~93% pure, with as many as 50 contaminants identified by gas chromatography (Lamoureux and Feil, 1980). Methoxychlor and four of the contaminants account for 98.88% of the technical grade product; the other contaminants represent <0.5% each. Only 25 of the contaminants have been rigorously or tentatively identified. Of the methoxychlor contaminants, Mazuch et al. (1975) identi- fied the reactive compound tetra cis(p-methoxyphenoxy)ethylene, and Mitchell et al. (1978) suggested that 3,6-dimethoxy-9,10-bis(p-methoxyphenyl)phenanthrene and 3,6,11,24-tetramethoxydibenzo(g,p)chrysene, which belong to the polycyclic aromatic class, many of which have been shown to have biological activity, may be present as contaminants. The mutagenic activity of these three compounds and purified methoxychlor was investigated by Grant et al. (1976).

Grant et al. (1976) used the Ames Salmonella assay with strains TA1535, TA1537, TA1538 and TA98 and a liver S-9 metabolic activation system from rats induced with either Aroclor or phenobarbital. The compounds were tested at doses of 2-200 μg/plate; at each dose, 0, 20, 50 and sometimes 150 μℓ of S-9 was used to be assured of optimal metabolic activation. Only the chrysene derivative was mutagenic, with the strongest response observed in strain TA98 with 50 μℓ of S-9 from phenobarbital-induced rats.

The doses used in this study were below the levels toxic to the bacteria; although the compounds other than the chrysene derivative did not appear to be mutagenic, higher concentrations could have been assayed. In addition, it cannot be ruled out that some of the many other contaminants of methoxychlor may be mutagenic, but that methoxychlor's toxicity to the bacteria may mask the activity in assays of the technical grade product.

Teratogenicity and Other Reproductive Effects

Teratogenic Effects. Khera et al. (1978) studied the teratogenicity and fetal toxicity of technical grade methoxychlor and a formulation of methoxychlor (50% methoxychlor and 50% unknown ingredients) in Wistar rats. Pregnant female rats in groups of 20 were given the test substances in corn oil at doses of 0, 50 (only with technical grade methoxychlor), 100, 200 and 400 mg/kg. The dams were gavaged on days 6 through 15 of gestation and killed on day 22. The only sign of toxicity in the dams discussed by the authors was a decrease in body weight gain during treatment for all dose levels. This decrease in body weight was still present at necropsy following removal of the uteri in animals treated with 100 and 200 mg/kg of the formulation product of methoxychlor and with 400 mg/kg of technical grade methoxychlor. The data presented did not appear to support this observation. If weight loss had persisted following removal of the uterus, this would have indicated that it had been due to an adverse effect on the dam rather than a reflection of a small litter size or poor fetal development.

Treatment of the dams with either technical grade methoxychlor or the formulation product reduced the number of rats with fetuses and the number of live fetuses per pregnancy at the higher dose levels (Table V-12) (Khera

TABLE V-12

The Prenatal Effects of Methoxychlor, Technical Grade and Formulation, Given to Rats by Intubation on Days 6 through 15 of Gestation[a]

	Methoxychlor, Technical Grade					Methoxychlor Formulation			
	Dose (mg/kg)					Dose (mg/kg)			
	0	50	100	200	400	0	100	200	400
Number of rats with live fetuses[b]	19	18	20	16	9	17	18	17	15
Number of corpora lutea per pregnancy[b]	13.3 ± 0.4	12.8 ± 0.6	13.3 ± 0.3	13.7 ± 0.7	11.4 ± 1.1	14.0 ± 0.5	13.9 ± 0.4	13.8 ± 0.5	13.5 ± 0.5
Number of live fetuses per pregnancy	12.2 ± 0.5	11.0 ± 0.8	11.6 ± 0.5	7.4 ± 1.2[c]	2.7 ± 1.4[c]	111.7 ± 0.8	12.1 ± 0.6	9.8 ± 0.8	5[c].0 ± 0.9
Dead fetuses and resorbed fetuses x 100/total implant sites	3	11	11	40[c]	70[c]	8	7	19[c]	60[c]
Fetal weight (g)[b]	5.2 ± 0.1	5.2 ± 0.1	4.9 ± 0.1	4.1 ± 0.4[c]	3.3 ± 0.8[c]	5.0 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.2 ± 0.5
Number of anomalous fetuses[d]/number examined	11/230	25/209	35/232	26/117[c]	16/24[c]	14/199	20/218	40/167[c]	25/75[c]
Number of anomalous litters/total examined	5/19	11/19	13/20	11/14	6/6	9/18	8/18	14/17	11/13

TABLE V-12 (cont.)

	Methoxychlor, Technical Grade					Methoxychlor Formulation			
	Dose (mg/kg)					Dose (mg/kg)			
	0	50	100	200	400	0	100	200	400
Anomalies (number of fetuses affected)									
Wavy ribs, uni- and bilateral	6	18	31	22	16	3	12	30	22
Extra ribs, uni- and bilaterial	3	6	3	2	NR	5	5	3	NR
Sternebrae nonopposed	NR	NR	NR	NR	NR	NR	NR	1	2
Sternebrae missing or unossified	NR	1	NR	NR	NR	NR	NR	NR	2
Calvarium, delayed ossification	2	5	NR	NR	NR	NR	NR	10	14
Bent scapula, radius, or ulna	NR	3	1	NR	5	NR	NR	3	2
Runted fetuses	2	NR	1	NR	1	NR	NR	NR	NR
Subcutaneous hematoma	NR	2	NR	NR	NR	NR	NR	NR	NR
Right atrium enlarged	NR	NR	NR	2	1	NR	NR	NR	NR
Encephaly	NR	NR	NR	NR	NR	NR	2	NR	NR

[a] Source: Adapted from Khera et al., 1978

[b] Mean ± S.E.

[c] $p<0.05$

[d] A fetus may have more than one anomaly.

NR - Not reported

et al., 1978). Another sign that methoxychlor was fetotoxic was the reduction in fetal weight observed at the two high-dose levels of technical grade methoxychlor. The incidence of fetal abnormalities was increased at the 200 and 400 mg/kg level, with skeletal abnormalities being most frequently observed. The skeletal abnormalities consisted of a sharp bend of the ribs, which occurred bilaterally. The authors proposed that methoxychlor arrests calcium deposition, especially in the ribs, with the result that physical forces can cause the bones to deform. The increased incidence of fetal abnormalities occurred only at doses that the authors claimed produced maternal weight loss, and the toxicity of methoxychlor to the dams may have adversely affected the maturation of the fetuses. Analyzing these data for dose-related trends might provide further information on the teratogenic or fetotoxic effects of methoxychlor.

Nonteratogenic Reproductive Effects. Certain steroid hormones are involved in reproduction, therefore, alteration of their rate or mode of action could affect the reproductive processes. Similarly, changes in the ability of the steroid to bind to its receptor could also alter the biological function of the steriod. Methoxychlor is apparently involved in two types of interactions with steriod hormones (Kupfer and Bulger, 1976). The first type involves the ability of methoxychlor to enhance the activity of certain hepatic enzymes in the MFO system. This, in turn, increases the level of activity of hydroxylases in the liver microsomes that metabolize endogenous steroids, thereby effectively reducing the concentration of circulating levels (see Target Organ Toxicity Section). On the other hand, methoxychlor manifests estrogen-like effects in mammals (Kupfer, 1975; Kupfer and Bulger, 1976).

Estrogenic Effects on the Female Reproductive System -- Moderate to severe mammary gland hyperplasia and secretion were observed in 16 of 24 female miniature swine following prolonged administration of a 1 g/kg bw/day diet of methoxychlor (Bierbower, 1966) (Tables V-13 and V-14). Other estrogen-like symptoms included swollen vulva and endometrial hyperplasia in the uteri of all animals (see Effects of Subchronic Exposure Section). Tegeris et al. (1966) and Stein (1970) also reported dose-dependent breast hyperplasia, milk secretion and hypertrophic uteri in Hormel miniature swine observed within 7 weeks of dietary administration of 1-4 g technical methoxychlor/kg bw/day. Corpora lutea failed to develop in female rats fed 100 or 200 mg methoxychlor/kg bw/day for 14 days before and continuously after mating (Bal and Mungkornkarn, 1977). This paper was unobtainable and therefore cited from IARC (1979); no other details were provided.

Reproductive effects observed by Harris et al. (1974) were not solely explained by the estrogenic effects of methoxychlor because of the pronounced inconsistencies between the methoxychlor and the estradiol benzoate (EB) that were fed to female rats in a parallel experiment as a positive control. Reproduction was not affected by a chow meal diet (no mention of another vehicle) of 1000 ppm technical methoxychlor (17-25 Sprague-Dawley rats 60-90 days old per group), but diets containing higher levels, 2500 and 5000 ppm fed 6 weeks before mating and throughout pregnancy and lactation, reduced the number of animals that mated. Persistent estrus was evident and only one rat littered. These effects proved to be reversible when adult rats were returned to untreated diets. The lowest level of EB in the diet (0.25 ppm) reduced the number of rats that mated and the litter size, but higher levels (0.62 and 1.25 ppm) did not. Female rats fed 1.25 ppm ED did

TABLE V-13

Some Studies Showing Estrogenic Effects in Experimental Animals Following Acute Oral Administration of Methoxychlor

Species/Strain	Sex	No.	Weight/Age	Vehicle	Purity	Dose/Duration	Effects	Reference
Mice/NIH general purpose	F[a]	10	10 g/NR	sesame oil	technical	0.5 mg total dose given over 3 days	117% increase in uterine weight over controls	Tullner, 1961
		9	9 g/NR			1.0 mg total dose given over 3 days	273% increase in uterine weight over controls	
		10	10 g/NR			5.0 mg total dose given over 3 days	563% increase in uterine weight over controls	
Rats/Sprague-Dawley	F[b]	10	54 g/NR	sesame oil	technical	20 mg total dose given over 3 days	192% increase in uterine weight over controls	Tullner, 1961
	F[c]	10	70 g/NR			20 mg total dose given over 3 days	241% increase in uterine weight over controls	
Rats/Sprague-Dawley	F	NR	NR/NR	5% ethanol in sesame oil (V/V)	unspecified (from Sigma Chemical Co.)	30 mg/kg bw/day on days 14-20 of gestation	Body weights of female offspring were significantly increased over controls ($p<0.001$); no effect on ovary, uterine or adrenal weight in offspring; no effect on litter size; 4 animals were anovulatory, but this was not statistically significant	Gellert and Wilson, 1979

[a] Ovariectomy

[b] Ovariectomy plus adrenalectomy

[c] Hypophysectomy

NR = Not reported

TABLE V-14

Some Studies Showing Estrogenic Effects in Experimental Animals Following Subchronic Oral Administration of Methoxychlor

Species/Strain	Sex	No.	Weight/Age	Vehicle	Purity	Dose/Duration	Effects	Reference
Rats/ Sprague-Dawley	F	17	NR/60-90 days	mixed directly into diet	technical (90% p,p'-methoxychlor)	1000 mg/kg diet fed for 6 weeks before mating, through pregnancy and lactation (offspring maintained on mother's diet after weaning for 6 weeks), pair-fed	No effect on reproduction in dams; decreased vaginal opening age by 41% in offspring, reduced mating in offspring and decreased reproductive performance in males (not completely reversible); decrease in ovarian weight by 33% and increase in liver weight, in virgin adults	Harris et al., 1974
		20				2500 mg/kg diet, same as above	Only 45% mated and 1/9 littered (effects reversible when treatment ceased); decrease in vaginal opening age by 51%; increase in uterine weight of 49% and decrease in ovarian weight of 66% in virgin adults	
		25				5000 mg/kg diet, same as above	8% mated but no rats littered or had implantation sites (effects reversible), constant estrus in dams after 5 weeks of treatment; increase in uterine weight of 20% and decrease in ovarian weight of 66% in virgin adults.	
Rats/ Sprague-Dawley	M	5-6/ group	37-41 g/NR	diet	technical grade	10,000 mg/kg diet x 33-55 days in pair-fed and nonpair-fed groups	Body weight loss, marked testicular atrophy, decreased weight of seminal vesicles, testes, and ventral prostate glands, increase in adrenal weight, cystic tubular nephropathy in kidneys, histopathologic changes in testes and kidneys but not in liver, pancreas, or adrenal glands.	Tullner and Edgcomb, 1962
Rats/NR	M-F	20/ group	average weight, 54 g/NR	diet	technical grade	100 mg/kg diet x 45 days	No effect on growth, survival, organ weight, or histopathologic examination of tissues.	Hodge et al., 1950

TABLE V-14 (cont.)

Species/Strain	Sex	No.	Weight/Age	Vehicle	Purity	Dose/Duration	Effects	Reference
Rats/NR (cont.)						1000 mg/kg diet x 45 days	Small but detectable growth depression, no effect on survival, organ weights, or histopathologic examination of tissues.	
				pair-fed group		10,000 mg/kg diet x 30 days	Marked decrease in testes weight (0.2 g vs. 1.8 g in controls) and development, histologic testicular atrophy, minor decreases in growth rate, red-blood cell counts, and hemoglobin, no histopathologic changes in any organs except testes.	
						30,000 mg/kg diet x 45 days	Marked growth retardation, 8/10 deaths in each of the male and female groups, histologic changes in testes, (but not in liver, kidneys, spleen, heart, stomach, lungs or brain), dramatic decreases in organ weights; effect on hematology.	
Rats/ Sprague-Dawley	M	12	322 g/ 76-91 days	diet	technical grade	2000 mg/kg diet x 90 days	Marked growth depression, significantly decreased testicular and ventral prostate weights, (11% decrease in testes weight, 23% decrease in body weight, and 40% decrease in ventral prostate weight)	Shain et al., 1977
Swine/ miniature	M-F	24 F 4 M	NR/6.5-23.5 months	diet	unspecified	1 g/kg bw/day for 6-40 weeks; (increased to 2 g/kg bw in 7 animals at 4-12 weeks)	2/28 deaths between 10-17 weeks; signs of continuous heat after 21 days, prolapsed vagina; swollen vulva, moderate to severe breast hyperplasia with secretion; pseudocyesis; endometrial hyperplasia in uteri of all females; histopathological examination revealed chronic interstitial renal fibrosis, in all treated pigs, uterine cysts, hypotrophic testes in 2/4 boars, moderate to severe fatty liver changes in 4 females, offspring had swollen vulvas and were in heat.	Bierbower, 1966

TABLE V-14 (cont.)

Species/Strain	Sex	No.	Weight/Age	Vehicle	Purity	Dose/Duration	Effects	Reference
Swine/ miniature	M castrated	4	NR/mature	diet	unspecified	1-2 g/kg bw/day* x 3.5 -5.5 months	Teats of all animals were maximally enlarged after 2.5 months (development of glandular tissue with secretory material in acini); chronic interstitial renal fibrosis.	Bierbower, 1967a
Swine/ miniature	M castrated	3	NR/11 weeks	diet	unspecified	2 g/kg bw/day x 18 weeks	Hyperplasia of the ducts in the mammary gland; (changes were less severe than with mature pigs) chronic interstitial renal fibrosis.	Bierbower, 1967b
Swine/Hormel miniature	M-F	6/group	25-45 kg/ 8 months	diet	technical grade	1.0 or 2.0 g/kg bw/day x 6 months or 4.0 g/kg bw/day x 4.5 months	Initial body weight loss in low- and mid-dose groups, then recovery, body weight loss throughout the study in high-dose group; in all groups; dose-dependent breast hyperplasia with milk secretion observed within 7 weeks, none came into estrus; excessively long hair on backs; dose-dependent elevation of blood urea nitrogen; histopathologic examination revealed advanced renal nephritis, (regardless of dose) hyperplastic and hypertrophic mammary glands, hypertrophic uteri, no effect on hematology no histopathologic changes seen in liver, thyroid, pancreas, adrenals, lungs, heart, stomach, large and small intestine, bladder, prostate, testes, muscle, spleen or bone marrow.	Tegeris et al., 1966

*Purity of methoxychlor unspecified

NR = Not reported

not exhibit persistent estrus. In female offspring, methoxychlor treatment of dams (1000 ppm) significantly hastened the onset of vaginal openings. Mating was reduced by prenatal and postweaning exposure of both female and male offspring to 1000 ppm methoxychlor. Prenatal influences of methoxychlor could not be completely reversed by feeding untreated diet at weaning, and as a result, only 50% of these rats littered. Uterine weights were increased in virgin adult female rats fed 2500 and 5000 ppm technical methoxychlor for 6 weeks (Harris et al., 1974). Ovarian weights were decreased 33% in the group fed 1000 ppm and 66% with the 2500 and 5000 ppm groups.

Methoxychlor failed to induce reproductive effects (such as persistent estrus, anovulation or tonic levels of serum estradiol) in male and female offspring following perinatal oral administration to dams of 30 mg/kg bw/day on days 14-20 of gestation (Gellert and Wilson, 1979). Tullner (1961) observed an increase in uterine weights of mice and rats in an animal care facility. Extensive examination of all environmental conditions revealed that a single dusting with an insectide had occurred 3 days previously. Each of the insecticide components was tested individually, and it was determined that methoxychlor (composition of 1.9%) had produced the effects observed in the female genital tract.

Effects on the Male Reproductive System -- Decreased testicular weight and histopathologic atrophy of the testes in pair-fed rats had been reported, but at a very high dietary level of technical methoxychlor; a 1% diet was fed for 30-55 days (Hodge et al., 1950; Tullner and Edgcomb, 1962).

Tullner and Edgcomb (1962) reported decreased weights of seminal vesicles and ventral prostate glands as well (see Effects of Subchronic Exposure Section). A significant increase in the number of degenerating stage VII spermatocytes was observed in rats fed a 5% methoxychlor diet for 7 days (Russell et al., 1981). Arrested spermatogenesis was observed in rats fed 100 or 200 mg methoxychlor/kg bw for 70 days (Bal and Mungkornkarn, 1977).

Prolonged feeding studies of 1-2 g/kg/day methoxychlor in miniature swine also resulted in reduced testes size and hypotrophic testicular atrophy (Bierbower, 1966). Pseudopregnancy and hyperplasia of the mammary gland ducts were observed in similar feeding experiments in mature and immature castrated swine (Bierbower, 1967a,b). Methoxychlor (0.5-4.0 mℓ/kg/day, 5 days/week for 109 days) produced dose-related atrophy of the testes in all surviving rabbits (7/7) when applied dermally as a 30% solution in dimethyl phthalate (Nelson, 1942). More detailed information on all of these studies can be found in the Effects of Subchronic Exposure Section.

Summary of Health Effects in Animals

In rats, the acute oral LD_{50} of methoxychlor, whether purified or technical grade, administered in lipid vehicle is ~6 g/kg bw (Hodge et al., 1950; Smith et al., 1946; Lehman, 1951). In other species, values for acute oral LD_{50} were reported as >2.0 g/kg bw for hamsters (Cabral et al., 1979), >2.5 g/kg bw for monkeys and 2.9 g/kg bw for mice (Coulston and Serrone, 1969). Symptoms of toxicity include depression of CNS, progressive weakness, diarrhea and death within 36-48 hours (Smith et al., 1946; Lehman, 1951). Rabbits receiving 200 mg/kg bw/day died after 4-15 doses and had histological changes in kidney, heart, liver and gall bladder (Smith et al., 1946; Lillie et al., 1947). Much lower single oral doses (0.04-0.64 g/kg bw) to rats produced changes in hepatic glycogen metabolism (Morgan and Hickenbottom, 1979). Methoxychlor was more toxic than DDT when administered intravenously to rats (Lehman, 1954).

The most commonly observed effect of sublethal oral chronic methoxychlor administration at dietary levels of 500-1000 ppm is growth retardation (Tullner and Edgcomb, 1962; Hodge et al., 1950; Shain et al., 1977; NCI, 1978), although results in pair-fed controls indicate that this is an organoleptic rather than a toxic effect (Tullner and Edgcomb, 1962). Dietary levels of 2000 ppm methoxychlor resulted in significantly decreased testicular weights in rats (Shain et al., 1977). Interspecies differences in response include the following: in rats, cystic tubular nephropathy (Hodge et al., 1950) and testicular atrophy (Tullner and Edgcomb, 1962; Shain et al., 1977; Hodge et al., 1950); in dogs, CNS responses (Tegeris et al., 1966); in swine, chronic nephritis (Stein, 1968), fatty changes in the liver, mammary gland hypertrophy and uterine cysts (Bierbower, 1966, 1967a,b). Histopathological changes in kidneys, livers and reproductive organs were not observed at dietary levels <1000 ppm.

Carbon tetrachloride increased the toxicity of methoxychlor when co-administered to rats (Laug and Kunze, 1951); however, chlorpromazine (Weiss and Brodie, 1968), Aramite, DDT and thiourea (Radomski et al., 1965; Deichmann et al., 1967) had no effect on methoxychlor toxicity or tumorigenicity.

Methoxychlor does not appear to be carcinogenic, mutagenic or teratogenic. No significantly increased tumor incidences were observed in bioassays in rats and mice when compared with controls (Nelson and Fitzhugh, 1951; Hodge et al., 1952, 1966; Radomski et al., 1965; Davis, 1969; Deichmann et al., 1967; NCI, 1978), although the NCI (1978) bioassay gave inconclusive results. Reanalysis of histological slides (Reuber, 1978, 1980) from the above studies indicated that there was a positive response and inconclusive results were obtained in later studies (Greisemer and Cueto, 1980; Harper et al, 1982). Negative results were obtained in mutagenicity assays with or without metabolic activation in bacterial and yeast systems, in the *Drosophila melanogaster* recessive lethal assay, in unscheduled DNA synthesis assays using primary rat hepatocyte and human fibroblast cultures, and in cell transformation assays (Ashwood-Smith et al., 1972; Probst et al., 1981; Simmon, 1979; Waters et al., 1982; Pienta, 1980; Traul et al., 1981; Dunkel et al., 1981); weakly positive transformation response was obtained in cultured BALB/3T3 cells (Dunkel et al., 1981). In a teratogenicity study (Khera et al., 1978), wavy ribs were observed in fetuses, but only at doses which were toxic to dams.

VI. HEALTH EFFECTS IN HUMANS

Effects of Acute Exposure

In humans (number unspecified), an acute oral dose of 2 mg technical methoxychlor/kg produced no abnormal histological changes in the liver, small intestine or testes, as revealed by electron micrographs of biopsy material (Coulston and Serrone, 1969). The lethal oral dose for humans was estimated to be 450 g (6.4 g/kg for a 70 kg man) methoxychlor if ingested at one time (Lehman, 1949; Tilemans and Dormal, 1952). Documentation of this estimate is not provided by either of the investigators but it is probably a reasonable estimate based on an LD_{50} in rats. The onset of symptoms, according to Lehman (1949), would begin between 24 and 72 hours after ingestion and death would occur between 2 and 4 days. Lehman (1948) also estimated that 169 g of methoxychlor would be a "dangerous single dermal exposure for man." Documentation was not provided by the investigator. These estimates have probably been made at the request of others unfamiliar with the field but in need of approximations as general guidelines and have been viewed historically as having been based on the known facts at that time. No information is available on acute human poisonings to substantiate these estimates.

A report from the Pesticide Incident Monitoring System (PIMS, 1981) stated that eight humans were involved in five incidents of over-exposure to methoxychlor alone. Seven were affected but not treated and one individual required medical attention. Sixty-three people were affected in 56 incidents of methoxychlor intoxication in combination with other compounds. Of these individuals, 10 were hospitalized, 18 received medical attention and 35 were affected but not treated. In one particular instance, three people on a city park crew developed symptoms of sore throat, rhinitis, cramps, fatigue and anorexia while spraying methoxychlor (vehicle unspecified) for mosquito control. These symptoms developed within a few days and continued for ~7 days. Anorexia is the only symptom that has been described for methoxychlor poisoning in studies with experimental animals (Smith et al., 1946) (see Table V-1). No other details were provided in these short reports of human poisoning.

Only one instance of human illness associated with acute exposure to methoxychlor has been reported in the literature, and the exposure was in combination with another pesticide so that a cause-effect relationship could not be identified. A 27-year-old male continued to suffer neurological impairment (including a bilateral profound hearing loss and residual, peripheral neuropathies in the extremities) for more than 6 years following an acute exposure to an aerosol insecticide (Harell et al., 1978). The product sold under the name ORTHO Orchard Spray and contained 15% methoxychlor and 7.5% malathion (no vehicle specified), an organophosphorus insecticide. The total exposure period was 15-20 minutes, during which time the subject was spraying fruit trees.

The onset of symptoms was unusually slow; symptoms of blurred vision and nausea did not appear until 8-9 hours later (Harell et al., 1978). Vomiting and diarrhea occurred on day 2, jaundice and continuous bilateral tinnitus on day 3, deafness on day 4 and renal failure on day 5. Renal and hepatic function tests showed severe impairment, and a liver biopsy revealed a hepatitis attributed to the insecticides. Peripheral neuropathies appeared shortly thereafter and continued to persist, even though a complete recovery from all other symptoms had occurred. Considering the relatively low order of mammalian toxicity to these pesticides and the slow appearance of symptoms, however, it is possible that this illness had an etiology unrelated to the brief pesticide exposure.

Manifestations of poisoning from malathion usually occur from 5 minutes to 3 hours after ingestion in humans, who may be more susceptible than experimental animals (Matsumura, 1975). Acute intoxication may be manifested as CNS signs, such as confusion and convulsions in severe cases. Incontinence and lacrimation are usual symptoms and, in cases of ingestion, gastrointestinal disturbances occur. Onset of symptoms with methoxychlor, which is usually slow, requiring 24 hours or more, is evidenced by CNS depression and gastrointestinal symptoms (Lehman, 1949; Smith et al., 1946). Hepatic necrosis and renal degenerative changes in experimental animals following very high acute oral doses of methoxychlor have been reported (Lillie et al., 1947), so that perhaps hepatic damage, caused by methoxychlor in this instance, prevented detoxification of malathion. Both compounds are lipid soluble and would be rapidly absorbed in aerosol form. Although the acute oral LD_{50} doses in rats for both methoxychlor and malathion are in the same range (up to 6 g/kg), methoxychlor is considered to be of relatively low toxicity to humans, while numerous cases of poisoning and death from ingestion of low doses of malathion have been reported. It was conjectured that possibly a synergistic effect had resulted from the combination of methoxychlor and malathion; however, a report by Keplinger and Deichmann (1967) on the acute oral LD_{50} toxicities of pesticide combinations states that there is an additive but not a synergistic effect of such a combination when administered to rats. Another consideration would be the toxic effects of the unknown impurities contained in either methoxychlor or malathion.

Effects of Subchronic Exposure

Two reports on the subchronic oral toxicity of methoxychlor in male and female volunteers are available (Wills, 1969; Stein, 1970). The doses of methoxychlor (purity unspecified) were the same in both instances (0.5, 1.0 and 2.0 mg/kg bw/day); one study was for 4-6 weeks (Wills, 1969) (Table VI-1) and the other for 6-8 weeks (Stein, 1970). In neither instance was there any effect on body weight or routine biochemical analyses, nor was any microscopic tissue damage seen in the five biopsy specimens taken from 3-6 subjects (see Tables VI-1 and V-4). Electron microscopic examination of all tissue specimens was negative. No effect on the endoplasmic reticulum was observed, but hepatic enzyme activity was not specifically examined in any of the subjects studied. The menstrual cycle of female volunteers was not affected (Wills, 1969), and the volume of ejaculation from males was normal (Stein, 1970). A shortening of the neck of the spermatozoa was observed in the testes by Wills (1969) but not by Stein (1970).

Some comments on the Stein (1970) review article have already been made (see Effects of Subchronic Exposure). The information on methoxychlor from Wills (1969) is contained in a book entitled Current Research on Persistent Pesticides and this particular chapter is followed by a discussion. Wills is asked whether he observed any effect on the endoplasmic reticulum during microscopic examination of the liver. The answer is negative for both the liver and the intestine but Wills states, offhandedly, that the only change that was observed was a shortening of the neck of the spermatozoa in the testes (no mention is made of this observation in the text) and that this could have resulted from an effect on mitochondria. No other details or comments are provided regarding the importance of this observation.

TABLE VI-1

Subchronic Oral Toxicity of Methoxychlor in Humans*

Sex	Number	Weight/ Age	Vehicle	Purity	Dose/Duration	Effects
M-F	8/group	NR	gelatin capsules	NR	0.5, 1.0 or 2.0 mg/kg/bw/day x 4-6 weeks	No effect on clinical chemistry measurements; glucose, blood urea nitrogen, uric acid, total cholesterol, Na,K, total protein, SGOT, SGPT, lactic dehydrogenase, alkaline phosphatase; acid phosphatase, no damage to small intestine, bone marrow, liver or testes as revealed by regular and electron microscopic examination biopsies 3-6 subjects depending on tissue involved (also from 3 controls); no effect on distribution of fatty acids in subcutaneous fat; no effect on menstrual cycle; a shortening of the neck in sperm.

*Source: Adapted from Wills, 1969

NR = not reported

Pertinent data regarding effects in humans following subchronic dermal and inhalation exposures could not be located in the available literature.

Effects of Chronic Exposure

Pertinent data regarding the effects of chronic exposure of methoxychlor in humans could not be located in the available literature.

Carcinogenicity

Pertinent data regarding the carcinogenic effects of methoxychlor in humans could not be located in the available literature.

Reproductive Effects

Little information is available on reproductive effects of methoxychlor on humans. Ingestion of 0.5, 1.0 or 2.0 mg/kg bw/day methoxychlor (purity unspecified) for 4-6 weeks had no effect on the menstrual cycle of female volunteers (Wills, 1969). The same dosing schedule for 6-8 weeks did not affect the volume of ejaculation in male volunteers (Stein, 1970), but a shortening of the neck of the spermatozoa found in the testes was observed (Wills, 1969). Histopathologic and electron microscopic examination did not reveal any testicular damage in biopsy specimens (see Effects of Subchronic Exposure).

Summary of Health Effects in Humans

An acute oral dose of 2 mg methoxychlor/kg in humans produced no abnormalities in biopsies of liver, small intestine and testes (Coulston and Serrone, 1969). Lehman (1949) estimated that 450 g (6.4 g/kg) of methoxychlor taken orally would cause death within 2-4 days. Reports of accidental exposure to methoxychlor alone or in combination with other chemicals (PIMS, 1981; Harrell et al., 1978) do not give adequate dose-response data. Symptoms of methoxychlor poisoning include CNS depression, gastrointestinal distress and local irritation to the nose and throat.

No abnormal gross physiological, biochemical or histological changes were observed in male and female volunteers given oral doses of <2.0 mg/kg bw/day for 4-8 weeks (Wills, 1969; Stein, 1970), although shortening of the neck of spermatozoa was observed by Wills (1969).

Pertinent data regarding the effects of chronic exposure and carcinogenicity of methoxychlor in humans were not located in the available literature.

VII. MECHANISM OF TOXICITY

Methoxychlor has been shown to have effects on a variety of biochemical and physiological functions, which may contribute to its toxicity.

Effects on Hepatic Mixed Function Oxidase

Methoxychlor has been shown to increase the activity of hepatic microsomal drug-metabolizing enzymes (Kupfer, 1968; Hart and Fouts, 1965) (Table VII-1). Methoxychlor is active as an enzyme inducer, but it is apparently capable of less marked effects than DDT (Hart and Fouts, 1965). The effects observed are dependent on a number of factors: route of administration, age, species, sex, dose, duration of dose and nutritional status of the animal (Kuntzman et al., 1964).

Measurement of Hexobarbital/Phenobarbital Sleeping Times. The duration of hexobarbital and phenobarbital anesthesia (sleeping time) is a direct reflection of the rate of hexobarbital and phenobarbital metabolism by the hepatic microsomal enzyme system (Street, 1969). Measurement of hexobarbital sleeping time, following a single intraperitoneal injection of 100 mg/kg methoxychlor (supplied by Geigy Chemical Co.; purity unspecified), indicated that mice were much less sensitive than rats to the hepatic microsomal enzyme enhancement of methoxychlor (Hart and Fouts, 1965).

Hexobarbital sleeping time was moderately decreased in Wistar rats as a result of three daily equitoxic oral doses of methoxychlor (purity unspecified) (Klinger et al., 1973). Doses were administered as 5, 10 and 20% of the acute oral LD_{50} for methoxychlor (in rapeseed oil) as determined by the authors; however, the exact doses administered were not specified (Table VII-1).

TABLE VII-1

Studies on Hepatic Mixed Function Oxidase Activity Following Oral Administration of Methoxychlor to Rats

Nature of Exposure	Species/ Strain	Sex/No.	Weight/ Age	Vehicle/Purity of of Methoxychlor	Dose/Duration	Effects	Reference
Acute	rats/ Wistar	M/ 8/group	60-80 g/ 30 days	rapeseed oil/purity unspecified	5, 10 or 20% of the acute oral LD_{50}/rat/day in 0.5 mℓ/100 g of rapeseed oil x 3 days (exact doses unspecified)	Hexobarbital sleeping time was decreased ~22, 60 and 70% respectively after 5 hours; amidopyrine-N-demethylation was increased ~50 and 150% at the mid- and high doses; ascorbic acid urine content increased ~100% at the low dose	Klinger et al., 1973
Acute	rats Wistar	M/ 6/group	80-130 g/ NR	diet/95% pure methoxychlor mixed directly in diet	200 mg/kg diet fed for 2 weeks	No significant induction of aniline hydroxylase, aminopyrine demethylase or hexabarbital oxidase	Den Tonkelaar and Van Esch, 1974
Acute	rats/ Sprague-Dawley	M/ 5/group	80-140 g/ NR	dioxane/NR	0 and 500 mg/kg by gavage, single dose 24 hours before death	Slight increase in liver aryl hydrocarbon hydroxylase (AHH) activity (543 pmol/mg protein compared with 454 pmol/mg protein for controls). Two-fold increase in intestinal AHH activity over control (25 vs. 11.9 pmol/mg protein)	Manis and Kim, 1980
Subchronic	rats/NR	NR/NR	NR/NR	diet/NR	500 and 2500 mg/kg diet x 4 weeks; 500 and 2500 mg/kg diet x 52 weeks	Body weight loss, decreased food intake, increased hexabarbital sleeping time; no effect on survival, cardiovascular parameters, liver weight, spontaneous motor activities, histopathologic examination	Weiss et al., 1970
Subchronic	rats/ Wistar	M and F/ 5/group	30-40 g/ 21 days	diet, 4% corn oil/ p,p'-methoxychlor (Dupont)	20 mg/kg diet fed for 30, 78, 240 or 350 days	Pentobarbital sleeping times were not statistically significantly decreased at any time in females, but were in males at 78 days; no significant methoxychlor residues found in fat, liver, heart or brain	Villeneuve et al., 1972

NR - Not reported

A 20 ppm diet of methoxychlor (E.I. Dupont de Nemours and Co.) fed to male and female Wistar rats for 30 days did not reduce pentobarbital sleeping time (Villeneuve et al., 1972). After 78 days on the diet, sleeping time was significantly reduced in male rats (~13%) but not in female rats; 240 days of feeding had no effect on sleeping time in males and 350 days had no effect on females. The authors felt that an explanation of these results was not readily apparent. No significant methoxychlor residues were found in fat, brain, liver or heart tissues after 350 days of feeding. Weiss et al. (1970), in fact, noted an increase in hexobarbital sleeping time in adult rats fed a diet containing 500 ppm methoxychlor (purity unspecified) for periods of both 1 month and 12 months. This information was published as an abstract; therefore, most of the details are not presented.

Measurement of Hepatic Enzyme Activity. In reactions characteristic of hepatic microsomal enzyme activity in rats (aniline hydroxylase, aminopyrine demethylase, hexobarbital oxidase), enzyme induction did not occur following a 2-week diet containing up to 200 ppm of 95% pure methoxychlor (Den Tonkelaar and Van Esch, 1974). Aminopyrine-N-demethylation activity was increased (~50 and 150% at the two highest doses) in rat liver supernatant as a result of three daily equitoxic oral doses (5, 10 and 20% of the acute oral LD_{50}) of methoxychlor (Klinger et al., 1973). Manis and Kim (1980) failed to find an induction of rat hepatic aryl hydrocarbon hydroxylase (AHH), following a single dose (by gavage) of 500 mg/kg bw of methoxychlor; however, activity of intestinal AHH was increased 2-fold.

The N-dealkylation of aminopyrine by rat liver microsomes was significantly stimulated 8 days (but not 1 day) after three daily intraperitoneal injections of 100 mg/kg methoxychlor (supplied by Geigy Chemical Co.; purity unspecified) in corn oil (Hart and Fouts, 1965). Methoxychlor (Dupont Chemical Co.; purity unspecified) was not an effective enzyme inducer in mice as evidenced by the measurement of NADPH oxidase activity, cytochrome P-450 activity and aniline hydroxylation following three daily intraperitoneal injections of 100 mg/kg methoxychlor (Abernathy et al., 1971). Nigg et al. (1976) found that only sulfoxidation (as measured by the oxidation of p-chlorothioanisole) was significantly elevated in mouse hepatic microsomes following five daily intraperitoneal injections of 100 mg/kg methoxychlor (99% purity) in olive oil. N-Demethylation and cytochrome P-450 levels were significantly depressed by ~18 and 20%, respectively.

Measurement of Ascorbic Acid Biosynthesis. Enhanced ascorbic acid biosynthesis as a result of increased metabolism of glucose by the glucuronic acid pathway has also been utilized as an index of inducer activity (Conney et al., 1967; Street and Chadwick, 1975). Methoxychlor was not a potent inducer of the hepatic microsomal enzyme system when an increase in ascorbic acid excretion in rats was used to compare methoxychlor with DDT (Chadwick, 1967). A diet containing 500 ppm methoxychlor (purity unspecified) fed to a group of female rats for 8 weeks did not enhance ascorbic acid output in the urine. Ascorbic acid excretion in urine was approximately doubled, however, in rats following three daily equitoxic oral doses of methoxychlor (Klinger et al., 1973). The actual doses and the purity of methoxychlor administered were not given in the work by Klinger et al. (1973).

Other Physiological Effects

Gershbein (1970) was interested in whether the administration of hepatic microsomal enzyme inducers, such as methoxychlor, would induce liver regeneration in partially hepatectomized rats. The extent of liver regeneration was not affected by a diet of 200 ppm methoxychlor (99% purity) fed to Holtzman or Charles River male and female rats for 10 days.

Methoxychlor appears to interfere with some energy-linked functions at the mitochondrial level (Abo-Khatwa and Hollingworth, 1974). In _in vitro_ studies, 95% pure methoxychlor inhibited the oxygen uptake of both state 3 and state 4 respiration in rat liver mitochondria at relatively high concentrations, 10^{-4} M.

In an _in vitro_ study on the effect of methoxychlor on the fragility of human erythrocyte membranes, no correlation was revealed between antihemolytic potency and neurotoxicity (O'Brien and Hilton, 1978). This study was undertaken because the action of DDT as a neuroexcitant has been attributed to a delayed shut-off of the sodium gate during the action potential. It was postulated that DDT and its analogs might affect membranes by gross interference with their internal structure. The view was generally taken by the investigators that the effects of DDT and its analogs upon the sodium gate in nerves was most likely related to a stereospecific interaction with the gate and was not attributable to physical change in the matrix within which the gate operates. It was conceded, however, that the interior of the axon membrane could be drastically different from erythrocyte membranes.

Methoxychlor appears to affect the hepatic storage of vitamin A in rats. Groups of nine female Wistar rats (28 days old; 80 g) were fed a diet of 100 ppm methoxychlor (p,p'-isomer, recrystallized) during development, reproduction and lactation for a total exposure of 92 days (Phillips and Hatina, 1972). No effect on hepatic storage of vitamin A in dams was observed, but total liver reserves of vitamin A were significantly reduced (10.9%, $p = 0.01$) in the weanlings. Nonetheless, the reduction in liver vitamin A was small and all weanlings had acquired significant stores, according to the authors. Dietary levels of 100 ppm methoxychlor (Sigma Grade II probably 95% pure, according to the 1981 Sigma Catalogue) or higher, fed for 16 weeks to 23-day-old Sprague-Dawley rats, reduced liver vitamin A storage (Davison and Cox, 1976). A slight reduction (not statistically significant) in storage of 7-13% occurred when 10 ppm methoxychlor was fed (see Effects of Subchronic Exposure in Chapter V).

Studies on the Estrogenic Mechanism of Action

An initial event in the mechanism of the estrogenic effects of methoxychlor is its competition with estrogen for binding sites on the cytoplasmic receptors in the uterus (Kupfer and Bulger, 1976). An intraperitoneal injection of 50 mg/kg of purified methoxychlor in immature rats 2 hours before an intraperitoneal injection of [^{3}H]-estradiol inhibited the uptake of labeled estradiol by the rat uteri *in vivo* (Welch et al., 1969). Methoxychlor caused a statistically significant decrease of 28% in [^{3}H]-estradiol uptake by the rat uterus, while diethylstilbestrol (DES, a structurally similar compound) resulted in a decrease of 61%.

The possibility that methoxychlor acts by a mechanism similar to that of estradiol, namely by binding to the cytosolic estrogen receptor, was investigated indirectly by determining whether methoxychlor added _in vitro_ would interfere with the binding of [^{3}H]-estradiol to rat uterine cytosol protein. Nelson (1974) observed that methoxychlor (though relatively inactive compared with _o,p_'-DDT) inhibited [^{3}H]-estradiol binding to rat uterine cytosol _in vitro_. A positive correlation between this _in vitro_ inhibition of estradiol binding and an increase in uterine weight _in vivo_ was recognized. Technical and purified methoxychlor, administered by intraperitoneal injection of 50 mg/kg to immature rats, caused an inhibition of 76 and 35%, respectively, of [^{3}H]-estradiol binding to rat uterine cytosol. Uterine weights increased 36% with technical methoxychlor and 29% with the purified preparation 6 hours after the injection.

Bulger et al. (1978b) observed a significant increase in uterine weight 7 hours after an intraperitoneal injection of 10 mg/rat of 99% pure methoxychlor in ovariectomized rats. Further evidence for the estrogenic action of methoxychlor was provided by a 136-fold increase in ornithine decarboxylase (ODC) activity in the uterus, mimicking the action of estradiol. Induction of uterine ODC activity was selected as a monitor of estrogenic activity because of its dramatic response, as compared with other indicators of estrogenic activity, and because of its important metabolic function at the subcellular level in the biosynthesis of spermidine and spermine (Kupfer and Bulger, 1976; Bulger et al., 1978a).

To eliminate the effect of endogenous estrogen, some investigators used ovariectomized animals to elucidate the estrogenic effects of methoxychlor.

Bulger et al. (1978b) demonstrated that both laboratory grade and purified methoxychlor maintained estrogenic activity in the ovariectomized rat. Tullner (1961) also demonstrated a uterotropic effect of technical methoxychlor, as evidenced by an increase in uterine weight, when ovariectomized mice were administered a total oral dose of 0.5, 1.0 and 5.0 mg, and rats (both ovariectomized plus adrenalectomized, and hypophysectomized) were given a total oral dose (intubation with sesame oil) of 20 mg/animal during a 3-day period. These observations are in contrast to the findings of Fisher et al. (1952) that methoxychlor (synthesized in the investigators' laboratory) did not support estrus in ovariectomized rats with a total dose up to 45 mg (route of administration unspecified).

Estrogenic Contaminants and/or Biotransformation. Both *in vivo* and *in vitro* observations indicated that technical methoxychlor exhibited more pronounced estrogenic effects than purified preparations, suggesting that methoxychlor was contaminated with a material possessing estrogenic properties (Bitman and Cecil, 1970; Nelson, 1974). Technical methoxychlor contains as many as 50 contaminants, and it has been reported recently that chlorotrianisene, a potent estrogen, is present as a result of the commercial production process (Mitchell and West, 1978). The presence of this compound has been determined by mass spectroscopic analysis but it has not yet been quantitated (see Table II-1). It is safe to say, however, that methoxychlor would contain <0.5% chlorotrianisene (Lamoureux and Feil, 1980). This compound has been used clinically as a lactation inhibitor. It also has antifertility properties and results in a pronounced storage of estrogen in fat. DES is structurally related to this contaminant.

Technical methoxychlor exhibited four times greater estrogenic activity than the pure *p,p*'-isomer, as measured by the 18-hour glycogenic response of the immature rat uterus following a subcutaneous injection of 8 mg/rat (Bitman and Cecil, 1970). Bulger et al. (1978c) found that washing laboratory grade methoxychlor with aqueous base (5% sodium hyroxide) drastically reduced the ability of that methoxychlor to interfere with the *in vitro* binding of [^{3}H]-estradiol to receptors in rat uterine cytosol. The base-soluble fraction obtained from this wash, however, did cause a suppression of [^{3}H]-estradiol binding. It was concluded that technical methoxychlor contained an acidic, probably phenolic, contaminant that was potentially estrogenic. Because of the presence of residual estrogenic activity in the base-washed methoxychlor at high concentrations, however, it was conceivable that methoxychlor itself had an intrinsic binding affinity for the estrogen receptor, but that it was masked by a contaminant with a much greater affinity for the receptor (Bulger et al., 1978c). In a subsequent report, Bulger et al. (1980) found that highly purified methoxychlor had no effect on binding of [^{3}H]-estradiol-17 to rat uterine cytosolic estrogen receptor, while both laboratory grade methoxychlor (99% pure) and isolated impurities inhibited this binding. Pure methoxychlor had no effect on the distribution of the cytosolic and the nuclear estrogen receptors, while estradiol reduced the former receptor to 5% and elevated the latter receptor to 280% of the control levels. In addition, a major *in vivo* metabolite of methoxychlor (Kapoor et al., 1970), 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE or DMDE), had a very high affinity for the estrogen receptor (Bulger et al., 1978c).

Although some methoxychlor would be dehydrochlorinated to HPTE, the pseudo-first order rate constant for this reaction is 3.8×10^{-5} sec^{-1} at 27°C and pH 13 (extrapolated from higher temperatures) (Wolfe et al., 1976). This leads to a half-life of ~5 hours; since the extraction more than likely took less time, the amount of methoxychlor lost would probably be minimal. Acidic, phenolic contaminants have been identified and were listed in Table II-1.

Following studies on the ability of the mono- and bisphenol (HPTE) metabolites of methoxychlor to inhibit estradiol binding to rat uterine cytosol _in vitro_, it was suggested that the estrogenic effects of methoxychlor _in vivo_ were primarily due to metabolism tc the monophenol[2-(_p_-methoxyphenyl)-2-(_p_-hydroxyphenyl)-1,1,1-trichloroethane], which resembles DES in its mode of action (Ousterhout et al., 1979; 1981).

Kupfer and Bulger (1979) incubated purified methoxychlor and HPTE with liver microsomes and an NADPH-generating system in the presence of rat uteri to observe the effects of metabolism on the distribution of the uterine estrogen receptor in the cytosol and in the nucleus. The enzymatic transformation of methoxychlor into its phenolic metabolites appeared to be necessary in order to effectively translocate the cytosolic estrogen receptor into the nucleus in a mechanism similar to steroidal estrogens. The metabolite, HPTE, markedly stimulated rat uterine ODC activity and increased uterine wet weight 6 hours after intraperitoneal injection of ovariectomized rats (Bulger et al., 1978b). Fisher et al. (1952) had already observed that HPTE supported estrus in ovariectomized rats.

Studies on the Anti-Androgenic Mechanism of Action

It has been postulated by a number of investigators that an estrogen receptor is also present in rat testicular cytosol, but not in seminiferous tubules (Brinkmann et al., 1972; Mulder et al., 1973, 1974). This possibility prompted Bulger et al. (1978d) to investigate the effect of base-washed methoxychlor (Bulger et al., 1978c) and HPTE on the binding of [^{3}H]-estradiol to the 8-9S estrogen-binding protein of rat testicular cytosol. Even though methoxychlor was estrogenic _in vivo_ in the female, it failed to inhibit estradiol binding; however, HPTE, also estrogenic _in vivo_, caused significant suppression of estradiol binding in a manner similar to that observed with rat uterine cytosol. It would appear that metabolic activation of the parent compound is necessary to effect binding to the testicular cytosol.

In an abstract, Schein et al. (1977), noted that methoxychlor (concentration and purity unspecified) did not interfere with the _in vitro_ binding of [^{3}H]-dihydrotestosterone to its cytosol receptor in the accessory sex organs, the liver, or the kidneys of castrated mice. In other _in vitro_ studies, technical methoxychlor was a relatively weak inhibitor of 5α-dihydrotestosterone binding to rat ventral prostate cytoplasmic and nuclear androgen receptors (25% inhibition at 10^4 M excess of methoxychlor) (Shain et al., 1977). A 90-day feeding study by the same investigators in rats with a diet containing 2000 ppm technical methoxychlor resulted in significantly diminished body, testicular and ventral prostate weight relative to controls (see Effects of Subchronic Exposure in Chapter V). Mean plasma testosterone concentration was not significantly altered by methoxychlor ingestion, indicating that changes in both androgen receptor and prostate homeostasis could be attributed to the action of the pesticide occurring in the ventral prostate. These paradoxical results indicate that there are multiple sites of pesticide action, and that _in vitro_ competitive androgen binding studies are ambiguous predictors of a pesticide's effects on prostate homeostasis and androgen receptors. These studies do suggest, however, that the rat ventral prostate may be a sensitive indicator of the possible deleterious effects of methoxychlor on the accessory organs of reproduction.

In _in vitro_ studies, methoxychlor (purity unspecified) significantly inhibited $[^3H]$-testosterone metabolism in rat liver microsomes at concentrations of 10^{-4} M but not at 10^{-6} M or less (Donovan et al., 1978). The inhibitory effect on steroid hydroxylation was somewhat less in mouse liver microsomes. Methoxychlor produced typical Type I spectral changes by interaction with hepatic microsomal cytochrome P-450. Double reciprocal plots of the spectral titration of methoxychlor binding to rodent liver microsomes showed that methoxychlor was bound to sites in both species with high affinity, but according to the authors, at concentrations $<2 \times 10^{-5}$ M, methoxychlor appeared to disrupt microsomal membranes, with subsequent loss of the Type I cytochrome P-450 spectrum.

In an investigation on the role of methoxychlor on altered imprinting during the critical period of rat development, Lamartiniere et al. (1982) studied the effect of neonatal exposure to methoxychlor (98% pure) on adult and prepubertal activities of sex-differentiated hepatic enzymes. Subcutaneous administration of 3 mg methoxychlor/rat on days 2, 4 and 6 postpartum resulted in significantly elevated ($p<0.01$) activities of hepatic monoamine oxidase in both male and female adult (63 days) rats, but not in prepubertal (20 days) males and females, when compared with controls. No changes were observed in glutathione S-transferase activity, UDP-glucuronyltransferase activity and cytochrome P-450 content. No significant changes were observed on adult or prepubertal testes, seminal vesicles, ventral prostate and body weight in male rats when compared with controls. Treatment of adult or prepubertal male rats with an equivalent dose schedule (3 x 333 mg/kg) did not result in altered monoamine oxidase activity. It was suggested that methoxychlor may have affected the brain hormone environment by binding to hypothalamic estrogen receptors and thereby altering the sex-differentiation of the enzyme.

Summary of Mechanism of Toxicity

Methoxychlor has a variety of biochemical and physiological effects, which may contribute to its toxicity.

Enhancement and depression of various hepatic mixed function oxidase activities in rats and mice following methoxychlor feeding have been reported (Klinger et al., 1973; Weiss et al., 1970; Villeneuve et al., 1972; Manis and Kim, 1980; Nigg et al., 1976). Methoxychlor inhibited oxygen uptake by rat liver mitochondria in vitro (Abo-Khatwa and Hollingworth, 1974), and reduced rat liver vitamin A storage in vivo (Davison and Cox, 1976).

Technical methoxychlor exerts its estrogenic effects by competing with estrogen for binding sites on cytoplasmic receptors (Kupfer and Bulger, 1976). This effect is actually due to estrogenic impurities or metabolites of methoxychlor, as evidenced by in vivo and in vitro studies comparing the action of technical and purified methoxychlor and metabolites of methoxychlor (Bulger et al., 1978c, 1980; Ousterhout, 1979, 1981). Evidence that methoxychlor and metabolites inhibit estrogen binding to receptors in rat testicular cytosol (Bulger et al., 1978d) and androgen binding to rat prostate cytosolic and nuclear androgen receptors (Shain et al., 1977) may partially explain the anti-androgenic effects of methoxychlor. The insecticide inhibited testosterone metabolism by rat liver microsomes in vitro (Donovan et al., 1978). Lamartiniere et al. (1982) found that subcutaneous administration of methoxychlor to neonatal rats resulted in elevated activities of hepatic monoamine oxidase (a sex-differentiated enzyme) in adults, possibly by binding to hypothalamic estrogen receptors during a critical period of rat imprinting.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the noncarcinogenic and carcinogenic effects. In the quantification of noncarcinogenic effects, an acceptable daily intake (ADI) is calculated. An adjusted acceptable daily intake (AADI) and health advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{\text{(NOAEL or LOAEL in mg/kg/day) (body weight in kg)}}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking water volume in } \ell\text{/day}} = \text{mg}/\ell$$

where: NOAEL = no-observed-adverse-effect level

LOAEL = lowest-observed-adverse-effect level

Body weight = 70 kg for adult or 10 kg for child

Drinking water volume = 2 ℓ/day for adults or 1 ℓ/day for children

Uncertainty/Safety Factor = 10, 100 or 1000

Utilizing these equations, the following drinking water concentrations are developed for noncarcinogenic effects:

1. A 1-day HA for 10 kg child
2. A 1-day HA for 70 kg adult
3. A 10-day HA for 10 kg child
4. A 10-day HA for 70 kg adult
5. A lifetime AADI for a 70 kg adult

The distinctions made between the HA calculations (items 1-4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1-2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon noncarcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivities to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS, 1977, 1980) are typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.
- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.
- An uncertainty factor of 1000 is used when limited or incomplete acute or chronic toxicity data are available or when only the acute or chronic toxicity data identify a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines (NRC, 1977) for selection of an uncertainty factor, but must be considered, include the following: 1) the quality of the toxicology data, 2) the significance of the adverse effect, and 3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen (there is insufficient evidence to classify methoxychlor as a carcinogen following oral exposure), mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical by drinking water. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for noncontinuous animal feeding, nonlifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 ℓ of water per day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as 1) incomplete knowledge concerning the health effects of contaminants in drinking water, 2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results, and 3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Existing Guidelines, Recommendations and Standards

Water. A maximum contaminant level of 0.1 mg/ℓ for methoxychlor in drinking water was promulgated in 1975 as an interim standard by the U.S. EPA (Federal Register, 1975a). The same maximum contaminant level has been established for bottled water by the FDA (Federal Register, 1975b).

NAS (1977) has suggested a NOAEL for methoxychlor in drinking water of 0.700 mg/ℓ, assuming that 20% of the total ADI (see Acceptable Daily Intake Section) comes from this source, or of 3.5 mg/ℓ, assuming that 100% of the total ADI comes from this source.

The WHO has recommended a drinking water criterion of 30 μg/ℓ for methoxychlor (WHO, 1984).

Acceptable Daily Intake. NAS (1977) has suggested an ADI for humans of 0.1 mg methoxychlor/kg bw based on a NOAEL for rats of 10 mg/kg bw/day. This NOAEL was estimated from a study in which rats were fed 200 ppm methoxychlor in the diet for 2 years, supposedly reported by Haag et al. (1959). The list of references for the NAS (1977) document, however, has a 1950 but not a 1959 reference for these authors. The 1950 paper contains no data regarding a chronic feeding study with rats and no pertinent 1959 paper authored by Haag and coworkers was found. The data could have been taken from the paper by Hodge et al. (1952), described in the Effects of Chronic Exposure Section in Chapter V of this document and not cited in the NAS (1977) document.

A temporary ADI for humans of 0-0.1 mg methoxychlor/kg bw was established by the Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues (WHO, 1975). This ADI was confirmed in 1977 (FAO/WHO, 1978). Both of these references were cited in IARC (1979), but the citations appear to contain some errors because no publications that corresponded exactly to these citations could be found and no information on methoxychlor was found in the reports that corresponded in part to these citations.

Raw Agricultural Commodities. Tolerance for residues of methoxychlor by the OPP (40 CFR 180.120; 1982) in or on raw agricultural commodities are given below:

> 100 parts per million in or on alfalfa, clover, cowpeas, grass for forage, peanut, forage, soybean forage. 14 parts per million in or on applies, apricots, asparagus, beans, beets (with or without tops) or beet greens alone, blackberries, blueberries (huckelberries), boysenberries, broccoli, brussell sprouts, cabbage,

carrots, cauliflower, cherries, collards, corn, cranberries, cucumbers, currants, dewberries, eggplants, gooseberries, grapes, kale, kohlrabi, lettuce, longanberries, melons, mushrooms, nectarines, peaches, peanuts, pears, peas, peppers, pineapples, plums (fresh prunes), pumpkins, quinces, radishes (with or without tops) or radish tops, raspberries, rutabagas (with or without tops) or rutabaga tops, spinach, squash, strawberries, summer squash, tomatoes, turnips (with or without tops) or turnip greens, youngberries.

7 parts per million in or on sweet potatoes and yams from preharvest and postharvest application.

3 parts per million in or on the fat of meat from cattle, goats, hogs, horses or sheep.

2 parts per million in or on the following grains from storage-bin treatment: Barley, corn, oats, rice, rye, sorghum gain, wheat.

1.25 parts per million in milk fat reflecting negligible residues in milk.

1 part per million in or on potatoes.

1 part per million in or on horseradish.

Air. The American Conference of Governmental Industrial Hygienists (ACGIH, 1980) adopted a TLV for methoxychlor in workplace air of 10 mg/m^3 as a TWA for an 8-hour workday. This value was thought to be low enough to avoid a health hazard to humans (ACGIH, 1979) on the basis of the low oral toxicity of methoxychlor to animals reported by Lehman (1952a), Hodge et al. (1952) and Tegeris et al. (1966).

OSHA (1976) has promulgated a standard for methoxychlor of 15 mg/m^3 as a TWA value for particulates in workplace air.

Noncarcinogenic Effects

Assessment of Acute Oral Data. No quantitative data on acute exposure of humans to methoxychlor were available. Much of the information on single oral exposures of laboratory animals to methoxychlor is concerned with lethality. For example, methoxychlor has a low order of toxicity: in rats, acute oral LD_{50}s of the insecticide in lipophilic vehicles have been estimated at ~6 g/kg bw (Hodge et al., 1950; Smith et al., 1946; Lehman, 1951). An LD_{50} value for mice was reported as 2.9±0.5 g/kg bw (Coulston and Serrone, 1969). Mortality of 50% was not observed at the highest doses tested for monkeys (2.5 g/kg bw) and hamsters (2.0 g/kg bw) (Coulston and Serrone, 1969; Cabral et al., 1979). Symptoms of toxicity include CNS depression, progressive weakness, diarrhea and death within 36-48 hours (Smith et al., 1946; Lehman, 1951). However, much lower single oral doses have been reported to produce changes in hepatic glycogen metabolism in rats, such as decreased lactate and glycogen phosphorylase and increased glucose-6-phosphatase (Morgan and Hickenbottom, 1979). These effects were observed in a group of seven male Holtzman rats after a single oral dose of 640 mg/kg bw of 90% pure methoxychlor in corn oil. Lower doses (i.e., 160, 40 or 10 mg/kg bw) were associated with fewer or no effects. Observations at all doses were not associated with pathologic changes in the liver and, therefore, all doses can be considered as NOELs or NOAELs.

Little et al. (1947) administered single doses of 2-8 g/kg of methoxychlor (purity and vehicle unspecified) to nine rats. Only one animal died, just 5 days after having received the lowest dose. Histopathological examination of this animal revealed many isolated hepatocytes in various stages of coagulative necrosis and fatty degeneration in the liver, kidney and heart muscle. Two rats sacrificed on day 7 also exhibited hepatic necrosis and small granulomas (dose unspecified). Fatty degeneration of isolated hepatic and renal cells, focal interstestial nephritis, small foci of interstitial myocarditis and pulmonary interstitial and perivascular infiltration were observed in the remaining animals.

Assessment of Short-Term Exposure Data. Toxicity data from short-term human exposure to methoxychlor are minimal. The only effect observed in humans was the reported shortening of the necks of spermatozoa when male volunteers were exposed to methoxychlor at doses up to 2 mg/kg bw/day by oral administration for 4-6 weeks (Wills, 1969). This effect was mentioned only in a question and answer period recorded at the end of the meeting paper and the details provided were insufficient to allow for the evaluation of these findings.

The only other information from human studies is provided in a report by Stein (1968). In this study volunteers were given methoxychlor at levels of 0.5, 1.0 or 2.0 mg/kg bw/day. No effects were reported for routine biochemical and hematologic studies or histopathology (both light and electron-micrographs) of the small intestine, testes or liver. This report, however, contained no information on experimental procedures, including number of subjects, age, or whether a matched control was used for comparision.

Loss of body weight, and growth retardation were the most frequent observations of sublethal oral toxicity of methoxychlor in laboratory animals (Tullner and Edgcomb, 1962; Hodge et al., 1950; Shain et al., 1977) after short-term exposures of up to 45 days. These effects were shown to be due to food refusal in pair-fed control experiments (Tullner and Edgcomb, 1962; Hodge et al., 1950) rather than to methoxychlor toxicity. Hodge et al. (1950) reported a small but detectable growth depression in groups of male and female rats (20/sex) fed methoxychlor at dietary concentrations of 1000 mg/kg for 45 days when compared with nonpair-fed controls. No effects on survival, organ weights, hematology or histological examination of tissues were found at this concentration. At higher dietary levels of methoxychlor (10,000-30,000 mg/kg diet for 30-55 days), testicular atrophy was observed in rats (Hodge et al., 1950).

Tullner and Edgecomb (1962) reported in rats (5-6 group) following oral administration of 10,000 mg/kg methoxychlor in diet for 35-55 days body weight loss, marked testicular atrophy, decreased weight of seminal vesicles and ventral prostate glands, cystic tubular nephropathy in kidneys, histopathological changes in testes and kidneys but not in liver, pancreas or adrenal glands.

In a study by Shain et al. (1979), there was marked growth depression, significantly decreased testicular and ventral prostate weight in the rats (12) following daily administration of 2000 mg/kg methoxychlor in the diet for 90 days.

Tegaris et al. (1966) studied the effects of methoxychlor in dogs following daily administration of 1000 mg/kg bw for a period of 6 months. The investigators observed in the dogs on methoxychlor (1) loss of body weight, (2) no effects on behavioral, survival time, hematology or clinical chemistry measurements histopathological damage in thyroid, pancreas, adrenals, lungs, liver, kidneys, prostate, testes, etc.

Assessment of Chronic Oral Data. The chronic animal studies (Hodge et al., 1952; NCI, 1978; Deichmann et al., 1967), designed primarily to assess the carcinogenicity of methoxychlor, provide toxicological data on nontumor pathology sufficient to determine adequate NOAELs. Subchronic data provide supporting evidence for the findings of the chronic studies. There are, however, no studies available that are specifically designed to evaluate both the histopathology and clinical chemistry of methoxychlor.

The most extensive nontumor pathologic examinations were performed by NCI (1978). Both rats (Osborne-Mendel) and mice (B6C3F1) were given methoxychlor in the diet in exposure groups consisting of 50 male and 50 female animals. The control group consisted of 20 animals of each sex. Two exposure levels were used, with the initial methoxychlor concentration being determined in a preliminary study. The concentration of methoxychlor was raised during the study as a result of the good tolerance of the high-dose groups to the experimental regime. The time-weighted concentrations in the diet for rats and mice are presented in Tables V-8 and V-10; they are 448 and 845 ppm for male rats, 750 and 1385 ppm for female rats, 1746 and 3491 ppm for male mice, and 997 and 1994 ppm for female mice.

The only gross, treatment-related, clinical sign of non-carcinogenic effects in the NCI (1978) bioassay was a dose-related decrease in body weight gain. The difference in body weight between the treated and control animals disappeared during the post-exposure observation period. [It had been demonstrated in shorter-term studies using pair-fed control rats that growth retardation represented an organoleptic rather than a toxic effect of methoxychlor feeding (Hodge et al., 1950; Davison and Cox, 1976).] No significant changes were noted in the NCI (1978) bioassay in any of the organs examined; therefore, all of these dietary concentrations can be considered as NOAELs. It should be noted, however, that not all animals in each group were examined histologically for changes in the reproductive system, a possible target organ system for methoxychlor as a result of its estrogenic action.

In an earlier chronic toxicity study by Hodge et al. (1952), rats (25 male and 25 female rats per group) were fed diets containing 0, 25, 200 and 1600 ppm methoxychlor for 2 years. The two low-dose groups had no adverse effects from treatment, while the high-dose animals had some retardation of weight gain. Sugar and protein values in the urine and hematological parameters were normal in all the treatment groups; however, other blood chemistries were not assessed. Histological evaluation of internal organs was not remarkable, but the liver was not included as an organ for routine examination. The retardation of weight gain at the high-dose level (1600 ppm) in conjunction with the lack of any other signs of toxicity following ingestion of methoxychlor would indicate that this dose level, as well as the lower two, represent NOAELs for lifetime methoxychlor exposure.

Lehman (1965) reported the results of a feeding study in rats (FDA, 1951) following administration of 0, 10, 25, 100, 200, 500 and 2000 mg/kg in diet of methoxychlor for 2 years. Growth retardation was observed at 200 ppm and above in animals. No histological damage attributable to the chemical was noted in animals over a 2-year period. A no-effect level of 100 ppm (5 mg/kg) was identified in this study.

A chronic study by Deichmann et al. (1967) revealed some histologic changes (hydropic swelling, granular cytoplasms, slight fatty metamorphosis, small focal areas of centrilobular necrosis and passive congestion in liver) in rats fed recrystallized methoxychlor in the diet at 1000 ppm. The experimental groups consisted of 30 Osborne-Mendel rats of each sex maintained on the diet for 27 months. Histological changes in the liver consisted of hydropic swelling, granular cytoplasm, slight fatty metamorphosis, small focal areas of centrolobular necrosis and passive congestion. Changes in the kidney were slight infiltration of lymphocytes in the cortex and very slight congestion. Changes in the spleen consisted of moderate to marked congestion in the pulp with hemolysis, giant follicles, lymphoreticular hyperplasia and small foci of myeloic metaplasia. This dietary concentration represents an adverse effect level (AEL).

As mentioned before, a subchronic study by Shain et al. (1977) reported a decrease in testicular weight in rats (12 animals/group) following a 90-day exposure to a dietary level of 2000 ppm methoxychlor. This level also represents an AEL.

Quantification of Noncarcinogenic Effects

Derivation of 1-Day HA. Accepting the 640 mg/kg bw dose from the Morgan and Hickenbotton (1979) study as the highest NOAEL, a 1-day HA is determined as follows:

For a child:

$$\text{1-day HA} = (640\ \text{mg/kg} \times 10\ \text{kg}) \div (1000 \times 1\ \ell) = 6.4\ \text{mg}/\ell$$

where 640 mg/kg = highest NOAEL based on the measurement of glycogen metabolism

10 kg = weight of a child

1000 = uncertainty factor representing two 10-fold decreases in dose for both intra- and interspecies variability to the toxicity of methoxychlor since specific information is not available and an additional 10-fold decrease in dose due to the limited scope of the data (i.e., only a few biochemical analyses were performed).

1 ℓ = volume of drinking water consumed per day by a 10 kg child.

This HA is equivalent to 6.4 mg/day or 0.64 mg/kg/day.

For an adult:

1 day HA = (640 mg/kg x 70 kg) ÷ (1000 x 2 ℓ)
= ~22 mg/ℓ

where 640 mg/kg = highest NOAEL based on the measurement of glycogen metabolism

70 kg = weight of an adult

1000 = uncertainty factor representing two 10-fold decreases in dose for both intra- and interspecies variability to the toxicity of methoxychlor since specific information is not available and an additional 10-fold decrease in dose due to the limited scope of the data (i.e., only a few biochemical analyses were performed.)

2 ℓ = volume of drinking water consumed/day by a 70 kg adult.

This HA is equivalent to 44.8 mg/day or 0.64 mg/kg/day.

Derivation of 10-Day HA. The dietary concentration of 1000 mg/kg diet/day (50 mg/kg bw, assuming a rat consumes 5% of its body weight as food per day) which produced slight growth retardation after short-term exposure (Hodge et al., 1950) represents a NOAEL, and can be used to estimate a 10-day HA. Using the same assumptions as in the 1-day HA calculation but an uncertainty factor of 100 (two 10-fold decreases in dose for both intra- and interspecies variability), a 10-day HA is derived as follows:

For a child:

10-day HA = (50 mg/kg bw/day x 10 kg) ÷ (100 x 1 ℓ/day)
= 5.0 mg/ℓ

This HA is equivalent to 5.0 mg/day or 0.50 mg/kg/day.

For an adult:

10-day HA = (50 mg/kg bw/day x 70 kg) ÷ (100 x 2 ℓ/day)
= 18 mg/ℓ

This HA is equivalent to 35 mg/day or 0.50 mg/kg/day.

In close agreement with the above calculated 10-day HAs are the values obtained when the available human data (Stein, 1968) are used for deriving a comparable 10-day HA (i.e., the highest NOAEL of 2.0 mg/kg bw/day) with the use of a 10-fold uncertainty factor for intraspecies variability. The resulting 10-day HAs are estimated as follows:

For a child:

10-day HA = (2.0 mg/kg bw/day x 10 kg) ÷ (10 x 1 ℓ/day)
= 2.0 mg/ℓ

This HA is equivalent to 2.0 mg/day or 0.20 mg/kg bw/day.

For an adult:

10-day HA = (2.0 mg/kg bw/day x 70 kg) ÷ (10 x 2 ℓ/day)
= 7.0 mg/ℓ

This HA is equivalent to 14 mg/day or 0.20 mg/kg bw/day.

Since the 10-day HA values of 2.0 mg/ℓ and 7.0 mg/ℓ for a child and an adult, respectively, are based on human data (Stein, 1968), these values should be considered for 10-day HAs.

Derivation of Lifetime AADI. The 845 ppm (42.3 mg/kg/day) dietary level producing growth retardation in male rats (NCI, 1978) in addition to insignificant but dose-related histological changes in the spleen of treated animals, is considered as the lowest-observed-effect level. The 100 ppm (5 mg/kg/day) dietary level producing no growth retardation in rats (Lehman, 1965) represents the highest NOAEL for lifetime methoxychlor exposure. Thus, the ADI is calculated as follows:

$$\text{ADI} = \frac{(5.0\ \text{mg/kg/day} \times 70\ \text{kg})}{100} = 3.5\ \text{mg/day}$$

where:

5.0 mg/kg/day = the NOAEL in rats (100 ppm) by Lehman (1965)

70 kg = weight of an adult

100 = uncertainty factor for both intra- and interspecies variability to the toxicity of methoxychlor

Using 3.5 mg/day as the ADI, the AADI is calculated as follows:

$$\text{AADI} = 3.5\ \text{mg/day}/2\ \ell\text{/day} = 1.7\ \text{mg}/\ell$$

where 2 ℓ day = the assumed daily drinking water consumption of a 70 kg adult

A summary of the data used to calculate HAs and AADIs in the Noncarcinogenic Effects Section is provided in Table VIII-1.

In general, the available data on the health effects of methoxychlor ingestion do not provide definitive threshold levels of systemic toxicity. It is apparent that methoxychlor does not exert its effects on any specific target organ below dietary levels at which the animals refuse to eat. The consequent growth retardation confounds the assessment of other systemic toxic effects that may be occurring. The values derived for HAs and AADIs

TABLE VIII-1

Summary of Data Used to Derive HA or AADI

Criteria	Dose (mg/kg bw/day)	Duration	Effect	Value of HA or AADI		Reference
				Child	Adult	
1-Day HA	640	single exposure	altered glycogen metabolism in rats	6.4 mg/ℓ (6.4 mg/day or 0.64 mg/kg bw/day)	22 mg/ℓ (44.8 mg/day or 0.64 mg/kg bw/day)	Morgan and Hickenbottom, 1979
10-Day HA	50	45 days	growth retardation in rats	5.0 mg/ℓ (5.0 mg/day or 0.50 mg/kg bw/day)	18 mg/ℓ (35 mg/day or 0.50 mg/kg bw/day)	Hodge et al., 1950
10-Day HA	2	4-6 weeks	routine biochemical and hematologic studies or histopathology of small intestine, testes and liver	2.0 mg/ℓ (2.0 mg/day or 0.20 mg/kg bw/day)	7.0 mg/ℓ 14 mg/day or 0.20 mg/kg bw/day)	Stein, 1968
Lifetime AADI	5	2 years	growth retardation in rats	NA	1.7 mg/ℓ (3.5 mg/day or 0.05 mg/kg bw/day)	Lehman, 1965

NA = Not applicable

are intended to represent estimates of the concentrations of methoxychlor in drinking water that will not cause adverse effects after exposures for 1 day, 10 days, longer-term and lifetime exposures.

In addition, it should be noted that these values exceed the solubility of methoxychlor (0.10-0.12 mg/ℓ at 25°C), in water as reported in the published information.

Assessment of Other Noncarcinogenic Health Effects

Only a single study (Khera et al., 1978) is available to assess whether methoxychlor is a teratogen. In this study, the treatment-related effects on the fetus (wavy ribs) were present only at doses that were toxic to dams. This abnormality was considered to be the result of disturbed maturation of the fetus rather than an indication of the presence of terata.

The effects of methoxychlor on the reproductive system (see Effects of Subchronic Exposure and Effects of Chronic Exposure Sections in Chapter V and see also Chapter VII) were considered in the derivations of the longer-term HA and lifetime AADI; however, little discussion of these studies was presented because the effects occurred at higher exposures than those used to derive the safe levels.

Assessment of Carcinogenicity Data

Methoxychlor has been tested for carcinogenicity in a number of studies using both rats and mice (Deichmann et al., 1967; Hodge et al., 1952, 1966; Radomski et al., 1965; NCI, 1978). Statistically significant increases in tumor incidences were not observed in any of these studies. Although Reuber

(1978, 1979a,b, 1980), after re-evaluation of the data, asserts that methoxychlor is carcinogenic, the conclusion of both NCI (1978) and IARC (1979) is that methoxychlor is not an animal carcinogen.

The *in vitro* mutagenicity assays of methoxychlor in the bacteria, *E. coli* and *S. typhimurium*, and in the yeast, *S. cerevisiae*, were negative both in the presence and absence of a metabolic activation system obtained from rat liver (Ashwood-Smith et al., 1972; Simmon, 1979). Other short-term assays of genotoxicity, unscheduled DNA syntheses, recessive lethal assay in *D. melanogaster*, and induction of DNA damage in DNA repair-deficient strains of *E. coli* and *B. subtilis*, were also negative. The study of Grant et al. (1976), suggesting the possible presence of the mutagenic contaminant, 3,6,11,14-tetramethoxydibenzo(g,p)chrysene, in methoxychlor, and the weakly positive transformation response of methoxychlor in cultured BALB/3T3 cells (Dunkel et al., 1981), do not present sufficient evidence to support a Carcinogenic potential for the compound.

Special Considerations

Multiple Pollutant Exposures. Rats treated orally with a combination of carbon tetrachloride (0.5-1.0 mℓ/kg) and methoxychlor (dose unspecified) developed tremors characteristic of DDT poisoning: it was suggested that carbon tetrachloride produced liver damage which resulted in impaired methoxychlor metabolism (Laug and Kunze, 1951; Winteringham and Barnes, 1955). Dietary administration of methoxychlor either alone or in combination with Aramite, DDT or thiourea to rats for 2 years produced no treatment related effects on mortality, food consumption, weight gain or tumor incidence (Radomski et al., 1952; Deichmann et al., 1967). In one case of human exposure to an aerosol insecticide containing 15% methoxychlor and 7.5% malathion, neurological impairment (hearing loss and peripheral neuropathies) continued for 6 years in a man after a 15-20 minute acute exposure (Harell et al., 1978).

High Risk Subpopulations. Workers involved in the manufacture, formulation and distribution of methoxychlor and agricultural workers may be exposed to potentially toxic concentrations of the insecticide. Methoxychlor is widely used for both crop and animal pests and is moderately persistent. Residues of methoxychlor have been detected in household dust since it is available for use in home gardening. Infants breast-fed by mothers with substantial methoxychlor residues in milk may be at increased risk based on a finding of increased activity of sex-differentiated enzymes in adult rats that were exposed neonatally to methoxychlor (Lamartiniere et al., 1982).

Summary

Several standards for methoxychlor in drinking water exist: maximum level promulgated by the U.S. EPA 0.1 mg/ℓ (Federal Register, 1975a); by the FDA for bottled water, 0.1 mg/ℓ (Federal Register, 1975b); by NAS (1977), NOAEL of 0.70 mg/ℓ. For dietary ingestion a suggested ADI for humans is also 0-0.1 mg methoxychlor/kg bw (NAS, 1977; WHO, 1975; FAO/WHO, 1978). Tolerances for methoxychlor residues on food range from 1 ppm on potatoes and horseradish to 100 ppm on forage crops (Code of Federal Regulations, 1980). The TLV-TWA for methoxychlor in workroom air adopted by the ACGIH (1980) is 10 mg/m^3 while OSHA (1976) promulgated a TLV-TWA of 15 mg/m^3. Special groups at risk include industrial and agricultural workers occupationally-exposed and breast fed infants.

In general, the available data on the health effects of methoxychlor ingestion do not provide definitive threshold levels of systemic toxicity. It is apparent that methoxychlor does not exert its effects on any specific target organ below dietary levels at which the animals refuse to eat. The consequent growth retardation confounds the assessment of other systemic toxic effects that may be occurring. The values derived for the 1-day HA, 10-day HA and AADI are intended to represent estimates of the concentrations of methoxychlor in drinking water that will not cause adverse effects after exposures for 1 day, 10 days, and longer-term and lifetime exposures, respectively.

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